

Evaluation of Conventional Karyotyping, Lactate Dehydrogenase Levels, White Blood Cells count, and Bone Marrow Blast Percentage as Good Prognostic Tests in Patients Diagnosed with Acute Myeloid Leukemia

Abdulkader Memeh^{1,*}, Abdel Galil Ghriwati¹, Khalid Khanji², Ibrahim Kebbe War³

¹Dept. of Biotechnology Engineering, Faculty of Technology engineering, Aleppo University

¹Dept. of Biotechnology Engineering, Faculty of Technology engineering, Aleppo University

²Dept. of Hematology, Faculty of Medicine, Aleppo University

³Dept. of Biochemistry and Clinical, Faculty of Pharmacy, Ebla Private University

* Corresponding author: Tel: (+963) 998554016, E-mail: ph.d.abdulkader.memeh@gmail.com

Received: 4 May 2023 / Accepted: 22 Jun 2023 / Published online : 3 July 2023

ABSTRACT

Acute myeloid leukemia (AML) is a kind of hematologic malignancy in which overall survival (OS) may be affected by many factors such as the patient's age, gender, conventional karyotyping (CK) findings, lactate dehydrogenase (LDH) concentration, white blood cells (WBC) count, and bone marrow blast (BMB) percentage at diagnosis. In this study, we aimed to evaluate the prognostic significance of those characteristics on OS at diagnosis. The current study included 98 AML patients who have been examined for cytology, CK, and immunophenotyping. The average patient's age was 39 years old. In addition, around 56% of patients had a chromosomal abnormality. Furthermore, the patients with LDH levels higher than 450 IU/L, WBC counts over 50 X10⁹/L, BMB% more than 50%, and AML-M0 had a shorter OS compared to other groups. However, the results suggested that CK, LDH, WBC count, and BMB% might be used as good prognostic tests in patients with AML at diagnosis.

Keywords: Acute myeloid Leukemia, Karyotyping, LDH, WBC, Bone marrow percentage

Running title: Evaluation of Conventional Karyotyping, LDH Levels, WBC Count, and BMB percentage as a Good Prognosis test in patients with AML

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy defined by abnormalities in myeloid cells lineage differentiation¹. It is more common in adult patients with acute leukemia; it is three times more common in adults than in adults with acute lymphocytic leukemia (ALL)².

The French-American-British (FAB) classification classified AML into eight subgroups (M0-M7) based

on the degree of differentiation of leukemia cells³.

Conventional karyotyping (CK) is a commonly used technique to identify both numerical and structural abnormalities in chromosomes. It can detect various types of structural abnormalities such as deletions, duplications, inversions, insertions, and balanced or unbalanced translocations⁴.

CK and molecular research show that gene mutations predominate in cytogenetically normal AML, whereas chromosomal alterations drive disease development and progression in aberrant karyotype AML⁵.

Characteristic chromosome abnormalities are diagnosed as essential for disease classification and are a significant element of the 2016 World Health Organization (WHO) classification⁶.

In AML, chromosomal abnormalities occur in the majority of leukemia cells, indicating acquired genomic modifications that may be diagnostic and prognostic⁷.

The cytogenetic risk factors in AML patients' include⁶⁻⁸:

- Favorable risk factors:
t(15;17)(q24;q21),
t(16;16)(p13.1;q22);
t(8;21)(q22;q22.1); t(1;11)(q21;q23);
mutated NPM1 without FLT3-ITD;
double CEBPA mutation, or GATA1 mutation.
- Intermediate risk factors: t(9;11)(p21.3;q23.3), loss chromosome Y, and Cytogenetic abnormalities not classified as favorable or adverse.
- Unfavorable risk factors: 3q abnormalities, -5/5q, -7, hypodiploid, complex karyotypes, TP53 mutation, inv(3)(q21.3q26.2), t(4;11)(q27;q23), t(5;11)(q35;p15.5), t(6;9)(p23;q34), t(6;11)(q27;q23), t(8;16)(p11.2;p13.3),

t(9;22)(q34.1;q11.2), or t(10;11)(p13;q23).

Lactate dehydrogenase (LDH) function is modified in most cancer cells as compared to normal cells. Even in the presence of oxygen, cancer cells employed LDH to improve their aerobic metabolic pathway (glycolysis, ATP synthesis, and lactate creation)⁹.

LDH may be easily detected in peripheral blood and it is widely used as a biomarker in the diagnostic setting of certain hematological malignancies¹⁰, such as multiple myeloma (MM), non-hodgkin lymphoma (NHL), myelodysplastic syndrome (MDS), hodgkin lymphoma (HL), AML, and acute lymphoblastic leukemia (ALL)¹¹⁻¹³.

It is not clear whether the increased serum levels of LDH commonly found in cancer patients reflect greater production and release of the enzyme by malignant cells¹⁴. A relationship between neoplasia and increased LDH levels has been reported by some studies in both human and animal tumors¹³.

An increase in the total of white blood cells (WBCs) count is more prevalent in acute leukemia than in chronic leukemia. Younger age, acute myeloid leukemia, the microgranular type of acute promyelocytic leukemia, acute lymphoblastic leukemia, and various chromosomal abnormalities are risk factors. Although it can affect any organ system, symptoms are most

commonly associated with the involvement of the brain, pulmonary, and renal microvasculature. The term "leukostasis" refers to "symptomatic hyperleukocytosis," which is a medical emergency that requires rapid detection and treatment to avert renal and respiratory failure or cerebral bleeding. The fundamental processes of hyperleukocytosis and leukostasis remain unknown¹⁵.

Hyperleukocytosis, previously known as a white blood cell count of more than $50 \times 10^9/L$, has been related to a poor prognosis due to early death and an increased probability of relapse¹⁶. On the other hand, compared to patients without hyperleukocytosis, a patient with hyperleukocytosis was correlated with higher rates of disseminated intravascular coagulation (DIC), and tumor lysis syndrome (TLS)¹⁷.

The percentage of immature cells in a patient's blood or bone marrow sample is known as blasts. In leukemia, the blast percentage is an important indicator of disease severity and progression. A high blast percentage indicates that there are more immature or abnormal cells, which might interfere with normal blood cell development. The blast percentage is frequently expressed as a fraction of the total number of blast-identified cells in the sample. The blast percentage is an important factor in assessing a patient's prognosis and

is used in the diagnosis, monitoring, and treatment of leukemia^{18,19}.

1. Materials and Methods:

1. Study design:

The current cohort study included patients who were newly diagnosed with AML according to the FAB classification and treated with standard leukemia chemotherapy (7+3) protocol for all AML subtypes except acute promyelocytic leukemia, which is treated with ATRA²⁰, between January 2019 and December 2022. Every person involved in this research had bone marrow aspiration, which was obtained using heparin and EDTA coagulation tubes to confirm AML diagnosis.

a. Ethical considerations:

Our research was accepted by the Ethical Committees of Aleppo University (Registration number /34/; date 7/1/2019). State that the study completed in agreement with the ethical international standards as that placed down in the 2010 Declaration of Helsinki and its advanced revisions with 1975. The patients in our study requested to provide written informed consent before enrollment.

b. Exclusion criteria:

Patients with secondary acute myeloid leukemia, mixed phenotypic acute leukemia (MPAL), MDS, or CK

examination that failed were excluded.

c. Data collection:

The current study included 98ii. patients attending to hematology department at Aleppo University Hospital and Ibn Al Rushd Hospital. The overall survival time and all doctors' observations have been obtained from patients' files during the follow-up period from the patient admission and follow-up office.

d. Measurements:

i. Hematological parameters:

The blast cells' morphological characteristics were examined using a cytology smear. The wedge smear technique was used to create blood films, with roughly 10 uL of blood put on one end of a glass slide (75X25 mm). The slide must be clean, dry, and grease-free. The spreader slide is then pulled back to touch the blood drop at a 30° angle in front of it. then smear was then treated with 100% ethanol and stained with Giemsa stain (HIMEDIA) to detect (Nucleus shape, chromatin type, and blast cell percentage), While the WBC count was identified by the Mindray BC-2800 hematology analyzer.

ii. Measurement of LDH concentration

Using a Mindray BS-300 analyzer and the lactate-to-pyruvate procedure with LDH (BioSystems) kit, the

serum LDH concentration was determined. The normal serum LDH reference range was 250-450 IU/L.

Flow Cytometric Immunophenotyping:

Flow cytometry was performed to immunophenotype blast cells collected samples. Single-cell suspensions (About 10⁶ cells/mL) were stained and were evaluated with four different fluorochrome-conjugated monoclonal antibodies CD45-APC, HLADr-PerCP, CD2-PerCp, CD3-FITC, CD4-PE, CD5-APC, CD7-FITC, CD8-PreCP, CD10-PE, CD19-PerCP, CD20-FITC, CD22-PerCp, CD23-APC, CD38-FITC, CD11b-FITC, CD13-PE, CD33-PerCp, CD14-PE, CD16-FITC, CD56-PE, CD34-APC, CD117-APC, CD163-FITC (Becton Dickinson Biosciences). Gently mixed, and incubated at room temperature for 30 minutes in the dark. Following that, the RBCs were lysed with a lysis solution (0.84 ammonium chloride, 0.12 gr potassium bicarbonate, and 0.002 tetrasodium EDTA in 100 mL distilled water). After 10 minutes of incubation at room temperature in the dark, the mixture was centrifuged for 5 minutes at 1200 rpm. Following the removal of the supernatant, the cells were treated with 0.5 mL of 2% paraformaldehyde solution before being evaluated with the BD FACSCanto (two lasers, six parameters) analyzer and data were

processed with BD FACSDiva™ software.

iv. Conventional karyotyping:

As a short-term culture, bone marrow aspiration samples were cultured in a flask with 10 mL of RPMI 1460 medium supplemented with (20% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate, and without mitogen). The flasks incubated for 48 hours at (37°C, 5% CO₂) before adding 100 uL of colcemid solution (10 ug/mL) and incubating for 60 minutes at 37°C. The culture then transferred to centrifuge tubes and centrifuged for 10 minutes at 1000 rpm. After removing the supernatants, the cells were resuspended in 10 ml of KCL solution (0.075 M) and incubated for 15 minutes at 37 °C. After centrifuging the sample tubes at 1000 rpm for 10 minutes, the cells were resuspended in fresh ice-cold fixative solution (acetic acid and methanol 1:3 ratio). The last procedure repeated until a leukocyte precipitate was obtained. The cells pellet re-suspended in 2 ml of fresh fixative solution, dropped onto a clean slide, and allowed to dry by warming. The G-banding technique was employed to stain chromosomes by immersing the slides in a 25% trypsin solution jar for 4 seconds, then in a phosphate buffer solution jar for 4 seconds, and finally in a 3% Giemsa stain

(HIMEDIA) for 5 minutes. CytoVision (version 3.92) software used to evaluate chromosomes in accordance with an international system for human cytogenomic nomenclature (2020). According to the results of CK all patients were classified into four groups (Normal idiogram, favorite risk, intermediate risk, or poor risk).

Statistical analysis

The mean ± standard deviation (SD) and the Kruskal-Wallis H test were used to compare continuous and categorical variables between groups, respectively. The Kaplan-Meier method was used to estimate survival curves, and to compare survival curves between groups. The groups were divided based on gender, age (<18 years versus >18 years), WBC count (<50 X10⁹/L versus >50 X10⁹/L), LDH value (<450 IU/L versus >450 IU/L), BMB% (<50% versus >50%), and CK results (Normal idiogram, favorite risk, intermediate risk, or poor risk). The data were analyzed using a multivariate Cox proportional hazards regression model to identify the risk variables for OS. The receiver operating characteristic (ROC) curve was created using the predicted probability of OS in patients with acute myeloid leukemia. The region under the ROC curve (AUC) was used to properly determine the overall

survival of conventional karyotyping, LDH concentration, WBC count, and bone marrow blast percentage. The IBM SPSS software (version 24) was utilized for statistical analysis. A significance level for analyses was a P value ≤ 0.05 .

2. Results:

a. Clinical features:

During the current study period, a total of 119 patients were diagnosed with AML. Cytology, conventional karyotyping, and immunophenotyping characteristics were investigated, 98 of them fulfilling the inclusion criteria **Figure 1**. The patients were distributed into 46 males and 52 females with an average age of 39 ± 24 years (range, 1- 85 years).

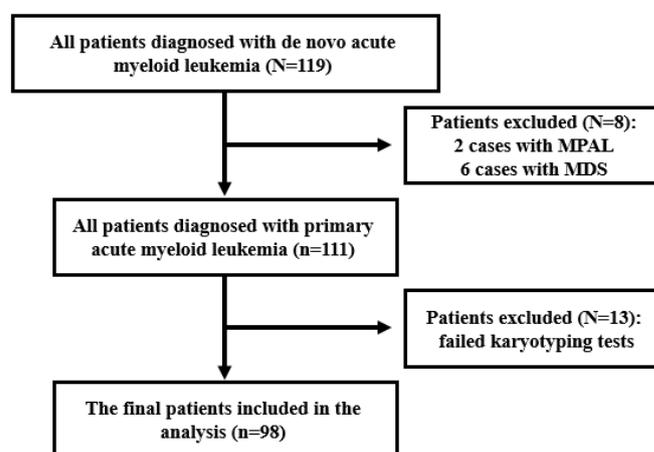
Figure (1): Illustration of patient exclusion criteria.
N: Number, MPAL: Mixed Phenotyping Acute Leukemia, MDS: Myelodysplastic Syndrome.

According to conventional karyotyping results, 43 patients (18 males versus 25 females) had a normal idiogram, 18 patients (10 males versus 8 females) had a favorite risk, 11 patients (7 males versus 4 females) had an intermediate risk, and 26 patients (11 males versus 15 females) had a poor risk **Figure 2**.

Figure (2): Patient distribution in the current research according to conversation karyotyping data.
t: Translocation, ph: polymorphism heterochromatin, Inv: Inversion, I: Isochromosome.

The statistical analysis showed significant differences between

conventional karyotyping subgroups' risk and acute myeloid leukemia FAB classification (P value < 0.000), LDH concentration levels (P value = 0.020), bone marrow blast percentage (P value = 0.022), and white blood cell count (P = value 0.015). However, there were no significant differences between conventional karyotyping subgroup risk and either patients' age (P value = 0.087) or gender (P value = 0.487) **Table 1**. Thus, lactate dehydrogenase, white blood cell



count, and blast percentage may be important risk biomarkers in individuals with acute myeloid leukemia.

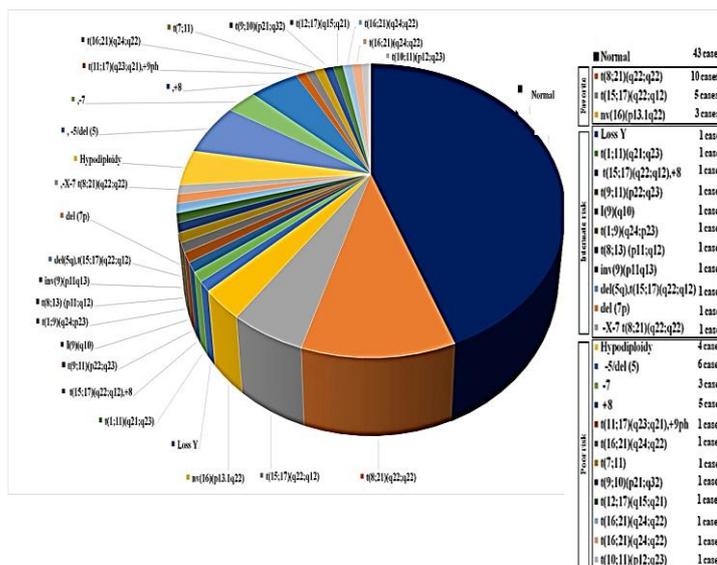


Table 1: Clinical characteristics of patients with Acute Myeloid Leukemia

Variable	Conventional Karyotype risk group				Total
	Normal	Favorite	Intermate	Poor	
Total	43	18	11	26	98
(%)	(43.9%)	(18.4%)	(11.2%)	(26.5%)	(100%)
Gender					
Male	18	10	7	11	46 (46.9%)
Female	25	8	4	15	52 (53.1%)
Age, Year					
18>	12	4	7	7	30 (30.6%)
18=	31	14	4	19	68 (69.4%)
AML subtypes according to FAB classification					
M0	1	0	0	5	6 (6.1%)
M1	5	0	2	3	10 (10.2%)
M2	11	2	2	7	22 (22.4%)
M3	0	14	3	0	17 (17.4%)
M4	2	2	2	2	8 (8.2%)
M5	24	0	2	8	34 (34.7%)
M6	0	0	0	1	1 (1.0%)
M7	0	0	0	0	0 (0.0%)
LDH levels					
450>	17	9	1	4	31 (31.6%)
450<	26	9	10	22	67 (68.4%)
Bone marrow blast (%)					
50% >	12	10	1	5	28 (28.6%)
50% <	31	8	10	21	70 (71.4%)
WBC (X 10⁹/L)					
50 >	13	12	2	7	34 (34.7%)
50 <	30	6	9	19	64 (65.3%)

AML: Acute Myeloid Leukemia, **LDH:** Lactate Dehydrogenase, **WBC:** White Blood Cell, **M0:** Undifferentiated acute myeloblastic leukemia, **M1:** Acute myeloblastic leukemia with minimal maturation, **M2:** Acute myeloblastic leukemia with maturation, **M3:** Acute promyelocytic leukemia, **M4:** Acute myelomonocytic leukemia, **M5:** Acute monocytic leukemia, **M6:** Acute erythroid leukemia, **M7:** Acute megakaryoblastic leukemia.

According to serum LDH concentration, 31 patients had an LDH concentration lower than 450 IU/L (13 males and 18 females). It

distributed according to conventional karyotyping subgroups risk to (17 cases with normal chromosomes, 9 cases with favorite risk, one case with intermediate risk, and 4 cases with poor risk). Otherwise, 67 cases had an LDH concentration higher than 450 IU/L (33 males and 34 females) that was distributed according to conventional karyotyping subgroups risk to (26 cases with normal chromosomes, 9 cases with favorite risk, 10 cases with intermediate risk, 22 cases with poor risk). Besides that, the current study found statistically significant variations between high serum LDH concentration in AML patients and the following characteristics: CK subgroups (P value= 0.020), WBC >50 X10⁹/L (P value< 0.000), BMB percentage >50% (P value< 0.000), and age (P value= 0.010). However, no significant difference was seen between LDH concentration with either FAB classification (P value= 0.219) or gender (P value= 0.522)

Table 2.

Table 2: The correlation between LDH concentration in patients with de novo AML and conventional karyotyping, BM blast (%), and WBCs.

Characteristics	Number (%)	LDH <450 U/L	LDH >450 U/L	p value
		31 (31.6%)	67 (68.4%)	
Gender				0.522
Male	46 (46.9%)	13	33	
Female	52 (53.1%)	18	34	
Age, Year				0.010
<18	30 (31.6%)	4	26	
>18	68 (68.4%)	27	41	
AML subtypes				0.219
M0	6 (6.1%)	1	5	
M1	10 (10.2%)	1	9	
M2	22 (22.4%)	9	13	
M3	17 (17.3%)	9	8	
M4	8 (8.2%)	2	6	
M5	34 (34.7%)	9	25	
M6	1 (1.1%)	0	1	
M7	0 (0.0%)	0	0	
Conventional karyotyping risk				0.020
Normal	43 (43.8%)	17	26	
Favorite	18 (18.4%)	9	9	
Intermediate	11 (11.2%)	1	10	
Poor	26 (26.6%)	4	22	
Bone marrow blast (%)				0.000
50% >	28 (28.6%)	18	10	
50% <	70 (71.4%)	13	57	
WBC (X 10⁹/L)				0.000
50 >	34 (34.7%)	26	8	
50 <	64 (65.3%)	5	59	

AML: Acute Myeloid Leukemia, LDH: Lactate Dehydrogenase, WBC: White Blood Cell, M0: Undifferentiated acute myeloblastic leukemia, M1: Acute myeloblastic leukemia with minimal maturation, M2: Acute myeloblastic leukemia with maturation, M3: Acute promyelocytic leukemia, M4: Acute myelomonocytic leukemia, M5: Acute monocytic leukemia, M6: Acute erythroid leukemia, M7: Acute megakaryoblastic leukemia.

b. Survival Analysis:

From January 2019 to December 2022, 98 patients were followed-up; the median follow-up period was 21.07 ±14.32 months (range, 2 days-47.1 months). At the end of the time of follow-up, 58 individuals were still surviving (59.2%). The Kaplan-Meier

statistical analysis results showed that there were statistically significant differences between overall survival time and conventional karyotyping groups' risk, LDH concentration, BMB percentage, white blood cells count, and FAB classification for AML. However, the patients with LDH concentration less than 450 IU/L, WBC count less than 50 X10⁹/L, and BMB percentage less than 50% had a longer overall survival time than those with LDH levels higher than 450 IU/L (30.64 versus 16.45, P value <0.000). WBC count higher than 50 X10⁹/L (31.26 versus 15.45, P value <0.000), and bone marrow blast cells higher than 50 % (31.89 versus 16.99, P value <0.000) **Table 3**. In addition, the present outcomes of this study showed that the overall survival time of patients with the favorite conventional karyotyping risk was longer than other conventional karyotyping groups' risk. There was the mean overall survival time (months) in patients with the favorite conventional karyotyping group risk (24.55±14.56) but it was around (15.80±14.33) for patients with the poor conventional karyotyping risk **Figure (3)**.

Figure (3): Relation between overall survival times and conventional karyotyping results (A), LDH concentration (B), Bone Marrow blast percentage (C), and white blood cells (D).

LDH: Lactate dehydrogenase, **BM:** Bone Marrow, **WBC:** White Blood Cell

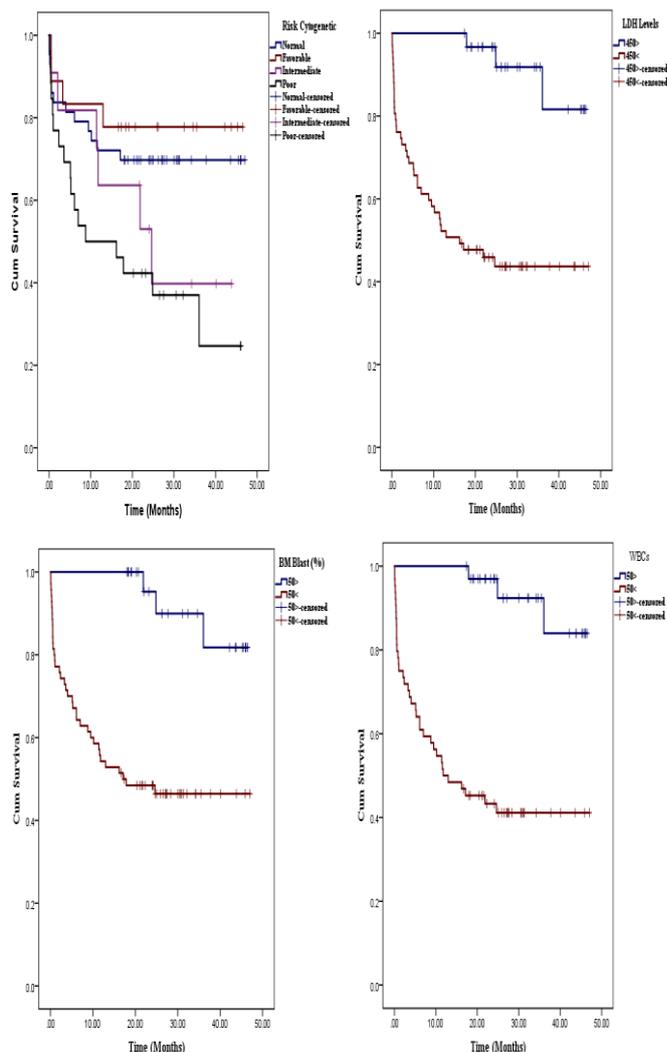
However, a multivariate Cox regression analysis was therefore conducted on the variables with P value <0.05.

Table 3: The correlation between overall survival times and conventional karyotyping, LDH concentration, BM blast%, and WBCs.

Variables	Number (%)	Overall Survival time (months) Mean ± SD	P value
Gender			0.341
Male	46 (46.9%)	22.57±13.63	
Female	52 (53.1%)	19.50±15.24	
Age, Year			0.353
<18	30 (30.6%)	20.72±14.43	
>18	68 (69.4%)	21.04±14.66	
AML subtypes			0.000
M0	6 (6.1%)	11.97±10.01	
M1	10 (10.2%)	18.16±15.82	
M2	22 (22.4%)	26.59±13.20	
M3	17 (17.3%)	23.48±15.99	
M4	8 (8.2%)	20.66±6.87	
M5	34 (34.7%)	18.55±14.63	
M6	1 (1.1%)	-	
M7	0 (0.0%)	-	
Conventional karyotyping			0.013
Normal	43 (43.8%)	22.40±14.18	
Favorite	18 (18.4%)	24.55±14.56	
Intermediate	11 (11.2%)	21.47±13.64	
Poor	26 (26.6%)	15.80±14.33	
Bone marrow blast (%)			0.000
50% >	28 (28.6%)	31.89±10.61	
50% <	70 (71.4%)	16.99±13.79	
LDH U/L			0.000
450 U/L >	31 (31.6%)	30.64±10.04	
450 U/L <	67 (68.4%)	16.45±14.18	
WBC (X 10⁹/L)			0.000
50 >	34 (34.7%)	31.26±10.24	
50 <	64 (65.3%)	15.45±13.53	

BM: Bone Marrow, **AML:** Acute Myeloid Leukemia, **LDH:** Lactate Dehydrogenase, **WBC:** White Blood Cell, **M0:** Undifferentiated acute myeloblastic leukemia, **M1:** Acute myeloblastic leukemia with minimal maturation, **M2:** Acute myeloblastic leukemia with maturation, **M3:** Acute promyelocytic leukemia, **M4:** Acute myelomonocytic leukemia, **M5:** Acute monocytic leukemia, **M6:** Acute erythroid leukemia, **M7:** Acute megakaryoblastic leukemia.

Table 4 shows results showed that independent adverse predictors of OS included the following factors: LDH concentration higher than 450 IU/L (Hazard factor [HR] = 0.071; 95% CI: 0.010-0.513; P value =0.009), bone



marrow blast percentage more than 50% (HR= 0.060; 95% CI: 0.008-0.434; P value =0.005) and WBC X10⁹/L (HR= 0.063; 95% CI: 0.015-0.260; P value =0.000).

Table 4: A multivariate analysis that affects overall survival in de novo AML patients.

Variables	Overall Survival Time (months)		
	P value	HR	(95% CI)
LDH U/L (450> versus 450<)	0.020	0.117	(0.036-0.382)
Blast% (50%> versus 50%<)	0.001	0.134	(0.041-0.437)
WBC X10 ⁹ (50> versus 50<)	0.000	0.098	(0.030-0.320)

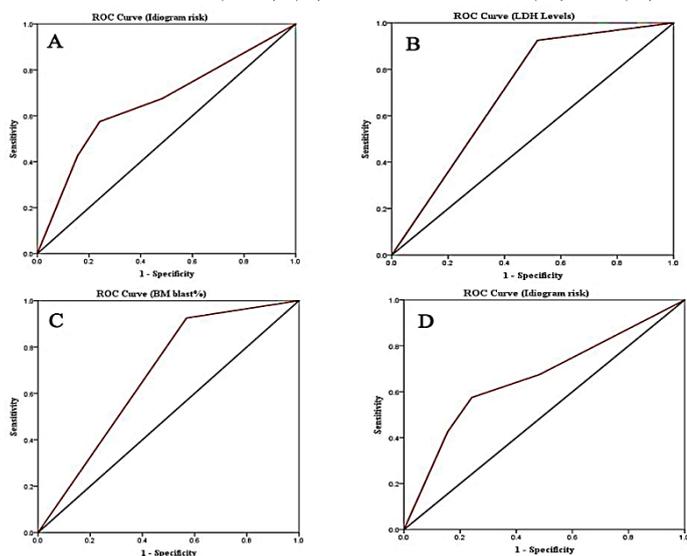
HR: Hazard Risk, CI: confidence interval, AML: Acute Myeloid Leukemia, LDH: Lactate Dehydrogenase, WBC: White Blood Cell.

karyotyping
AML: Acute Myeloid Leukemia, LDH: Lactate Dehydrogenase, WBC: White Blood Cell, BM: Bone Marrow.

c. Sensitivity and specificity analysis:

Receiver operating characteristic (ROC) curve analysis was used to assess the overall survival time performance of the conventional karyotyping group's risk, serum LDH concentration, WBC count, and BMB. The area under the ROC curve for conventional karyotyping groups risk was 0.660 (P value =0.007), LDH concentration was 0.704 (P value =0.001), WBC count was 0.730 (P value <0.000), and bone marrow blast cells percentage was 0.678 (P value =0.003) **Table 5**. The ROC curve was shown in **Figure 4**.

Figure (4): Receiver operating characteristic curves showed sensitivity and specificity for (A) Conventional karyotyping groups risk, (B) Lactate dehydrogenase concentration (IU/L), (C) Bone marrow blasts (%), and (D)



White blood cells count $\times 10^9/L$.

Table 5: Sensitivity and specificity analysis for conventional karyotyping, LDH concentration, BM blast%, and WBC count.

Variables	Area	P value
LDH (IU/L)	0.704	0.001
Blast (%)	0.678	0.003
WBC $\times 10^9/L$	0.730	0.000
Conventional	0.660	0.007

3. Discussion:

AML is a heterogeneous hematology malignancy, and the mechanism of leukemogenesis is still not fully known²¹. However, the majority of AML patients have acquired genetic abnormalities, including chromosomal abnormalities and somatic mutations, which may play roles in diagnosis and prognosis¹. Several studies have shown that cytogenetic abnormalities have prognostic value^{22,23}. The aimed of this study was to evaluate the prognostic significance of age, gender, CK groups' risk, serum LDH concentration, WBC count as well as BMB percentage in patients with de novo AML.

In this study, the average age of patients with AML was 39 years old, which is consistent with the median age at AML diagnosis in some studies^{24,25}. On the other hand, other research studies showed that the median age with AML diagnosis ranges between 63 to 71 years old^{26,27}, which may be associated with the general population's age in these countries.

According to the current study results, the most prevalent FAB classification subtype was AML-M5, which was found in 34 patients

(34.7%), which is consistent with some research studies^{28,29}. Other researchers showed that AML-M2^{30,31} and AML-M4^{32,33} subtypes are observed more frequently in patients with AML.

In addition, the conventional karyotyping of the blast cells is the strongest prognostic factor for survival³⁴. The current study also suggested that the AML-M0 subtype has the worse prognosis in AML subtypes, which is consistent with Walter, R study³⁵.

Conventional cytogenetics with banding techniques remains the gold standard for chromosomal abnormalities study in AML diagnosis³⁶. Nonrandom chromosomal abnormalities had been found by conventional cytogenetics in 60% of adults with AML⁷, acquired chromosomal abnormalities are observed in approximately 50-60% of individuals with de novo AML and are more prevalent in secondary AML^{28,36,37}. The current study's conventional karyotyping results showed that around 56% of patients diagnosed with de novo AML had a chromosomal abnormality. However, in this study, 13 cases (10.9%) of patients who were diagnosed with de novo acute myeloid leukemia had unsuccessful karyotyping, which is consistent with³⁸.

LDH is one of the most important enzymes of the anaerobic metabolic

pathway³⁹; it is playing an important role as a critical checkpoint of gluconeogenesis and deoxyribonucleic acid (DNA) metabolism⁴⁰.

Malignant cells have a unique metabolism that relies on the glycolytic sequence and the Krebs cycle to get their energy, thus the cells proceed to deplete glucose five to ten times faster than normal cells to convert it to lactate, and it was known as the Warburg effect⁹.

LDH levels are high in acute leukemia due to cell growth and the death of malignant cells. Elevated LDH is a poor prognostic marker for a variety of cancers, including acute leukemia³⁴. The current study found that patients with AML who had an increase in LDH concentration at diagnosis had a poorer prognosis than patients with normal LDH concentration. This is consistent with some studies that found a poor prognosis for patients with an elevated increase in LDH concentration at diagnosis^{12,34}.

In this research study, a WBC count of more than $50 \times 10^9/L$ was statistically associated with a poor prognosis (P value <0.000). Some study, on the other hand, has confirmed the poor prognostic impact of WBC count $>50 \times 10^9/L$ in Patients with AML^{29,41,42}. The results also showed of more than $50 \times 10^9/L$ was statistically associated with a

high LDH concentration and poor prognosis (P value <0.000), which also is associated with a poor prognosis, which is consistent with research⁴³. Although the causes of hyperleukocytosis are unknown, it has established that malignant blasts may adhere to the vascular endothelium and transmigrate into tissues⁴⁴.

Our results showed that an increased BMB count of <50% has been associated with an adverse prognosis (P value <0.000), consistent with research¹⁶. However, the mechanisms by which leukemic blast mobilization from and homing to the bone marrow occurs remain incompletely defined²³.

ROC statistical analysis was used to assess the prognostic ability of CK, LDH concentration, WBC count, and BMB (%) in patients with de novo acute myeloid leukemia, and ROC curves were created. The AUC for conventional karyotyping, LDH concentration, WBC count, and BMB (%) were 0.660, 0.704, 0.730, and 0.678, respectively, which might be used as good prognostic tests in patients with AML at diagnosis.

Acknowledgments

The KEBBE WAR Molecular and Cytogenetic Laboratory, and the Research Laboratory of the Faculty of Pharmacy at Aleppo University supported this research.

Reference:

- Quessada, J., Cuccuini, W., Saultier, P., Loosveld, M., Harrison, C. J., & Lafage-Pochitaloff, M. (2021). Cytogenetics of pediatric acute myeloid leukemia: a review of the current knowledge. *Genes*, 12(6), 924.
- Qu, H., Zheng, G., Cheng, S., Xie, W., Liu, X., Tao, Y., & Xie, B. (2020). Serum miR-22 is a novel prognostic marker for acute myeloid leukemia. *Journal of clinical laboratory analysis*, 34(9), e23370.
- Gregory, T. K., Wald, D., Chen, Y., Vermaat, J. M., Xiong, Y., & Tse, W. (2009). Molecular prognostic markers for adult acute myeloid leukemia with normal cytogenetics. *Journal of hematology & oncology*, 2(1), 1-10.
- Berisha, S. Z., Shetty, S., Prior, T. W., & Mitchell, A. L. (2020). Cytogenetic and molecular diagnostic testing associated with prenatal and postnatal birth defects. *Birth defects research*, 112(4), 293–306.
- Bochtler, T., Fröhling, S., & Krämer, A. (2015). Role of chromosomal aberrations in clonal diversity and progression of acute myeloid leukemia. *Leukemia*, 29(6), 1243–1252.
- Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., Bloomfield, C. D., Cazzola, M., & Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, 127(20), 2391–2405.
- Wiernik, Peter H.; Dutcher, Janice P.; Gertz, Morie A. (2018). Neoplastic Diseases of the Blood- Cytogenetics of Acute Leukemia.10.1007/978-3-319-64263-5 (Chapter 16), 281–306.

- Döhner, H., Wei, A. H., Appelbaum, F. R., Craddock, C., DiNardo, C. D., Dombret, H., Ebert, B. L., Fenaux, P., Godley, L. A., Hasserjian, R. P., Larson, R. A., Levine, R. L., Miyazaki, Y., Niederwieser, D., Ossenkoppele, G., Röllig, C., Sierra, J., Stein, E. M., Tallman, M. S., Tien, H. F., ... Löwenberg, B. (2022). Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*, 140(12), 1345–1377.
- Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells?. *Trends in biochemical sciences*, 41(3), 211–218.
- Geva, M., Pryce, A., Shouval, R., Fein, J. A., Danylesko, I., Shem-Tov, N., Yerushalmi, R., Shimoni, A., Szydlo, R., Pavlu, J., & Nagler, A. (2021). High lactate dehydrogenase at time of admission for allogeneic hematopoietic transplantation associates to poor survival in acute myeloid leukemia and non-Hodgkin lymphoma. *Bone marrow transplantation*, 56(11), 2690–2696.
- Kim, S. J., Hong, J. S., Chang, M. H., Kim, J. A., Kwak, J. Y., Kim, J. S., Yoon, D. H., Lee, W. S., Do, Y. R., Kang, H. J., Eom, H. S., Park, Y., Won, J. H., Mun, Y. C., Kim, H. J., Kwon, J. H., Kong, J. H., Oh, S. Y., Lee, S., Bae, S. H., ... Suh, C. (2016). Highly elevated serum lactate dehydrogenase is associated with central nervous system relapse in patients with diffuse large B-cell lymphoma: Results of a multicenter prospective cohort study. *Oncotarget*, 7(44), 72033–72043.
- Xiao, Z., Gong, R., Chen, X., Xiao, D., Luo, S., & Ji, Y. (2021). Association between serum lactate dehydrogenase and 60-day mortality in Chinese Hakka patients with acute myeloid leukemia: A cohort study. *Journal of clinical laboratory analysis*, 35(12), e24049.
- Hafiz, M. G., & Mannan, M. A. (2007). Serum lactate dehydrogenase level in childhood acute lymphoblastic leukemia. *Bangladesh Medical Research Council Bulletin*, 33(3), 88–91.
- Lehninger AL. Organ interrelationships in the metabolism of mammals. In: *Lehninger Textbook of Biochemistry*. New York: Worth; 2000. p. 829
- Korkmaz S. (2018). The management of hyperleukocytosis in 2017: Do we still need leukapheresis?. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*, 57(1), 4–7.
- Greenwood, M. J., Seftel, M. D., Richardson, C., Barbaric, D., Barnett, M. J., Bruyere, H., Forrest, D. L., Horsman, D. E., Smith, C., Song, K., Sutherland, H. J., Toze, C. L., Nevill, T. J., Nantel, S. H., & Hogge, D. E. (2006). Leukocyte count as a predictor of death during remission induction in acute myeloid leukemia. *Leukemia & lymphoma*, 47(7), 1245–1252.
- Bewersdorf, J. P., & Zeidan, A. M. (2020). Hyperleukocytosis and Leukostasis in Acute Myeloid Leukemia: Can a Better Understanding of the Underlying Molecular Pathophysiology Lead to Novel Treatments?. *Cells*, 9(10), 2310.

- DiNardo, C. D., Garcia-Manero, G., Pierce, S., Nazha, A., Bueso-Ramos, C., Jabbour, E., Ravandi, F., Cortes, J., & Kantarjian, H. (2016). Interactions and relevance of blast percentage and treatment strategy among younger and older patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). *American journal of hematology*, 91(2), 227–232.
- Sekar, M. D., Raj, M., & Manivannan, P. (2023). Role of Morphology in the Diagnosis of Acute Leukemias: Systematic Review. *Indian Journal of Medical and Paediatric Oncology*.
- Pagnano, K. B. B., Traina, F., Takahashi, T., Oliveira, G. B., Rossini, M. S., Lorand-Metze, I., ... & Souza, C. A. D. (2000). Conventional chemotherapy for acute myeloid leukemia: a Brazilian experience. *São Paulo Medical Journal*, 118, 173-178.
- Short, N. J., Rytting, M. E., & Cortes, J. E. (2018). Acute myeloid leukaemia. *Lancet* (London, England), 392(10147), 593–606.
- Short, N. J., Rytting, M. E., & Cortes, J. E. (2018). Acute myeloid leukaemia. *Lancet* (London, England), 392(10147), 593–606.
- Breems, D. A., Van Putten, W. L., De Greef, G. E., Van Zelderen-Bhola, S. L., Gerssen-Schoorl, K. B., Mellink, C. H., Nieuwint, A., Jotterand, M., Hagemeyer, A., Beverloo, H. B., & Löwenberg, B. (2008). Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 26(29), 4791–4797.
- Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., Dombret, H., Ebert, B. L., Fenaux, P., Larson, R. A., Levine, R. L., Lo-Coco, F., Naoe, T., Niederwieser, D., Ossenkoppele, G. J., Sanz, M., Sierra, J., Tallman, M. S., Tien, H. F., Wei, A. H., ... Bloomfield, C. D. (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*, 129(4), 424–447.
- Bekadja, M. A., Hamladji, R. M., Belhani, M., Ardjoun, F. Z., Abad, M. T., Touhami, H., ... & Midoun, N. (2011). A population-based study of the epidemiology and clinical features of adults with acute myeloid leukemia in Algeria: report on behalf of the Algerian Acute Leukemia Study Group. *Hematology/oncology and stem cell therapy*, 4(4), 161-166
- Philip, C., George, B., Ganapule, A., Korula, A., Jain, P., Alex, A. A., ... & Mathews, V. (2015). Acute myeloid leukaemia: challenges and real-world data from India. *British journal of haematology*, 170(1), 110-117.
- Shysh, A. C., Nguyen, L. T., Guo, M., Vaska, M., Naugler, C., & Rashid-Kolvear, F. (2018). The incidence of acute myeloid leukemia in Calgary, Alberta, Canada: a retrospective cohort study. *BMC public health*, 18(1), 1-5.
- Gangatharan, S. A., Grove, C. S., P'ng, S., O'Reilly, J., Joske, D., Leahy, M. F., ... & Wright, M. P. (2013). Acute myeloid leukaemia in Western Australia 1991–2005: a retrospective population-based study of 898 patients regarding epidemiology, cytogenetics, treatment and

- outcome. *Internal medicine journal*, 43(8), 903-911.
- Elnaggar, M. G., Mosad, E., Makboul, A., & Shafik, E. A. (2022). Cytogenetic profile of adult acute myeloid leukemia in Egypt: a single-center experience. *Molecular cytogenetics*, 15(1), 43.
- Jahic, A., Iljazovic, E., Hasic, S., Arnautovic, A. C., Sabitovic, D., Mesanovic, S., Sahovic, H., & Simendic, V. (2017). Prognostic Parameters of Acute Myeloid Leukaemia at Presentation. *Medical archives (Sarajevo, Bosnia and Herzegovina)*, 71(1), 20–24.
- Chang, F., Shamsi, T. S., & Waryah, A. M. (2016). Clinical and Hematological Profile of Acute Myeloid Leukemia (AML) Patients of Sindh. *J Hematol Thrombo Dis* 4: 239. doi: 10.4172/2329-8790.1000239 Page 2 of 5.
- Oliveira, L. C., Romano, L. G., Prado-Junior, B. P., Covas, D. T., Rego, E. M., & De Santis, G. C. (2010). Outcome of acute myeloid leukemia patients with hyperleukocytosis in Brazil. *Medical oncology (Northwood, London, England)*, 27(4), 1254–1259.
- Harani, M. S., Adil, S. N., Shaikh, M. U., Kakepoto, G. N., & Khurshid, M. (2005). Frequency of fab subtypes in acute myeloid leukemia patients at Aga Khan University Hospital Karachi. *Journal of Ayub Medical College, Abbottabad: JAMC*, 17(1), 26–29.
- Walaa Fikry, M. E. (2017). Lactate dehydrogenase (LDH) as prognostic marker in acute leukemia quantitative method. *J. Blood. Disord. Transfus*, 8, 1-8.
- Döhner, H., Estey, E. H., Amadori, S., Appelbaum, F. R., Büchner, T., Burnett, A. K., Dombret, H., Fenaux, P., Grimwade, D., Larson, R. A., Lo-Coco, F., Naoe, T., Niederwieser, D., Ossenkoppele, G. J., Sanz, M. A., Sierra, J., Tallman, M. S., Löwenberg, B., Bloomfield, C. D., & European LeukemiaNet (2010). Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*, 115(3), 453–474.
- Walter, R. B., Othus, M., Burnett, A. K., Löwenberg, B., Kantarjian, H. M., Ossenkoppele, G. J., Hills, R. K., van Montfort, K. G., Ravandi, F., Evans, A., Pierce, S. R., Appelbaum, F. R., & Estey, E. H. (2013). Significance of FAB subclassification of "acute myeloid leukemia, NOS" in the 2008 WHO classification: analysis of 5848 newly diagnosed patients. *Blood*, 121(13), 2424–2431.
- Schoch, C., & Haferlach, T. (2002). Cytogenetics in acute myeloid leukemia. *Current oncology reports*, 4(5), 390–397.
- Xu, J., Huang, B., Liu, X., Zhang, Y., Liu, Y., Chen, L., Luan, Y., Li, N., & Chu, X. (2017). Poor Prognosis in Acute Myeloid Leukemia Patients with Monosomal Karyotypes. *Monozomal Karyotipi Olan Akut Miyeloid Lösemili Hastalarda Kötü Prognoz. Turkish journal of haematology : official journal of Turkish Society of Haematology*, 34(2), 126–130.
- Medeiros, B. C., Othus, M., Estey, E. H., Fang, M., & Appelbaum, F. R. (2014). Unsuccessful diagnostic cytogenetic analysis is a poor prognostic feature in acute myeloid

- leukaemia. *British journal of haematology*, 164(2), 245–250.
- Farhana, A., & Lappin, S. L. (2022). *Biochemistry, Lactate Dehydrogenase*. In StatPearls. StatPearls Publishing.
- Holmes, R. S., & Goldberg, E. (2009). Computational analyses of mammalian lactate dehydrogenases: human, mouse, opossum and platypus LDHs. *Computational biology and chemistry*, 33(5), 379–385.
- Oliveira, L. C., Romano, L. G., Prado-Junior, B. P., Covas, D. T., Rego, E. M., & De Santis, G. C. (2010). Outcome of acute myeloid leukemia patients with hyperleukocytosis in Brazil. *Medical oncology (Northwood, London, England)*, 27(4), 1254–1259.
- Greenwood, M. J., Seftel, M. D., Richardson, C., Barbaric, D., Barnett, M. J., Bruyere, H., Forrest, D. L., Horsman, D. E., Smith, C., Song, K., Sutherland, H. J., Toze, C. L., Nevill, T. J., Nantel, S. H., & Hogge, D. E. (2006). Leukocyte count as a predictor of death during remission induction in acute myeloid leukemia. *Leukemia & lymphoma*, 47(7), 1245–1252.
- Alsulami, H. A., Alnashri, M. M., Bawazir, A. F., Alrashid, L. T., Dly, R. A., Alharbi, Y. A., & Qari, M. H. (2021). Prognostics and Clinical Outcomes in Patients Diagnosed With Acute Myeloid Leukemia (AML) in a Teaching Hospital. *Cureus*, 13(10).
- Bewersdorf, J. P., & Zeidan, A. M. (2020). Hyperleukocytosis and Leukostasis in Acute Myeloid Leukemia: Can a Better Understanding of the Underlying Molecular Pathophysiology Lead to Novel Treatments?. *Cells*, 9(10), 2310.

تقييم الصيغة الصبغية التقليدية ومستويات أنزيم نازع هيدروجين اللاكتات وتعداد كريات الدم البيضاء ونسبة الأرومات الدموية في نقي العظام كإختبارات تنبؤية جيدة لدى المرضى المُشخص لديهم الإصابة بالابيضاضات النقوية الحادة

عبد القادر ميمه¹، عبد الجليل غريواتي¹، خالد خانجي²، ابراهيم كبه وار³

¹قسم هندسة التقانات الحيوية، كلية الهندسة التقنية، جامعة حلب.

²قسم أمراض كلية الطب البشري، جامعة حلب.

³قسم الكيمياء السريرية والحيوية، كلية الصيدلة، جامعة ايبلا الخاصة.

المخلص

تُعد الابيضاضات النقوية الحادة (AML) إحدى أنماط الخباثات الدموية والتي قد يتأثر فيها زمن البقاء على قيد الحياة الكلي (OS) بالعديد من العوامل كعمر المرضى وجنسهم وكذلك كل من نتائج دراسة الصيغة الصبغية التقليدية (CK) ومستويات أنزيم نازع هيدروجين اللاكتات (LDH) وتعداد كريات الدم البيض (WBC) ونسبة الأرومات الدموية في نقي العظام (BMB) عند التشخيص. وقد هدفت الدراسة الحالية إلى تقييم أثر تلك العوامل على مدى استجابة المرضى على العلاج خلال فترة المتابعة. تضمنت الدراسة الحالية على 98 حالة من المرضى المُشخص لديهم الإصابة بالابيضاضات النقوية الحادة والذين تمت الحصول على نتائج الدراسات الخلوية والمناعية والصيغة الصبغية لهم، وقد أشارت نتائج الدراسة الحالية إلى أن متوسط عمر هؤلاء المرضى حوالي 39 سنة، بالإضافة إلى أنّ حوالي 56% من هؤلاء المرضى قد لوحظ لديهم وجود اضطرابات في الصيغة الصبغية. كما أشارت نتائج الدراسة الحالية إلى أنّ المرضى الذين لوحظ لديهم ارتفاع مستويات أنزيم نازع هيدروجين اللاكتات عن 450 وحدة دولية في اللتر، والمرضى الذين قد لوحظ لديهم ارتفاع تعداد كريات الدم البيض عن $50 \times 10^9/L$ ، والمرضى

الذين قد لوحظ لديهم ارتفاع نسبة الارومات الدمويّة عن 50% والمرضى الذين صنفوا من النمط AML-M0 قد لوحظ لديهم فترة أقل للبقاء على قيد الحياة. وبالتالي يمكن الاقتراح باعتبار اختبارات دراسة الصيغة الصبغيّة التقليديّة، ومستويات أنزيم نازع هيدروجين اللاكتات، وتعداد كريات الدم البيض، ونسبة الأرومات الدمويّة في نقي العظام من الاختبارات الإنذارية عند التشخيص لدى المرضى المصابين بالابيضاضات النقويّة الحادة.