

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

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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.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy defined by abnormalities in myeloid cells lineage differentiation [1]. 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 cells [3]. 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 translocations [4].

CK and molecular research show that gene mutations predominate in cytogenetically normal AML, whereas chromosomal alterations drive disease development and progression in aberrant karyotype AML [5]. Characteristic chromosome abnormalities are diagnosed as essential for disease classification and are a significant element of the 2016 World Health Organization (WHO) classification [6].

In AML, chromosomal abnormalities occur in the majority of leukemia cells, indicating acquired genomic modifications that may be diagnostic and prognostic [7].

The cytogenetic risk factors in AML patients' include [6-8]:

1) 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. 2) Intermediate risk factors: t(9;11) (p21.3;q23.3), loss chromosome Y, and Cytogenetic abnormalities not classified as favorable or adverse. 3) 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 malignancies [10], 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 cells [14]. A relationship between neoplasia and increased LDH levels has been reported by some studies in both human and animal tumors [13].

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 unknown [15].

Hyperleukocytosis, previously known as a white blood cells 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 relapse [16]. 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].

2. Materials and Methods

2.1 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 ATRA [20], 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.

2.2 Ethical consideration

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.

2.3 Exclusion criteria:

Patients with secondary acute myeloid leukemia, mixed phenotypic acute leukemia (MPAL), MDS, or CK examination that failed were excluded.

2.4 Data collection:

The current study included 98 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.

2.5 Measurements:

2.5.1 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.

2.5.2 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.

2.5.3 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 lyzed 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.

2.5.4 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 (37oC, 5% CO2) before adding 100 uL of colcemid solution (10 ug/mL) and incubating for 60 minutes at 37oC. 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).

2.6 Statistical analysis

The mean \pm 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 $\times 10^9/L$ versus >50 $\times 10^9/L$), 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 .

3. Results

3.1 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.

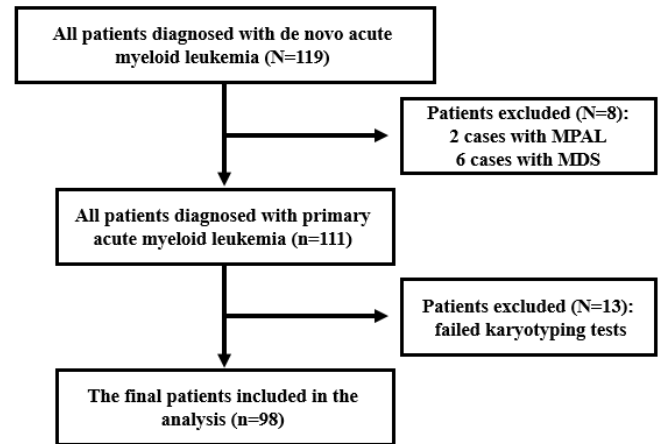


Figure 1: Illustration of patient exclusion criteria. N: Number, MPAL: Mixed Phenotyping Acute Leukemia, MDS: Myelodysplastic Syndrome.

The patients were distributed into 46 males and 52 females with an average age of 39 ± 24 years (range, 1- 85 years). 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.

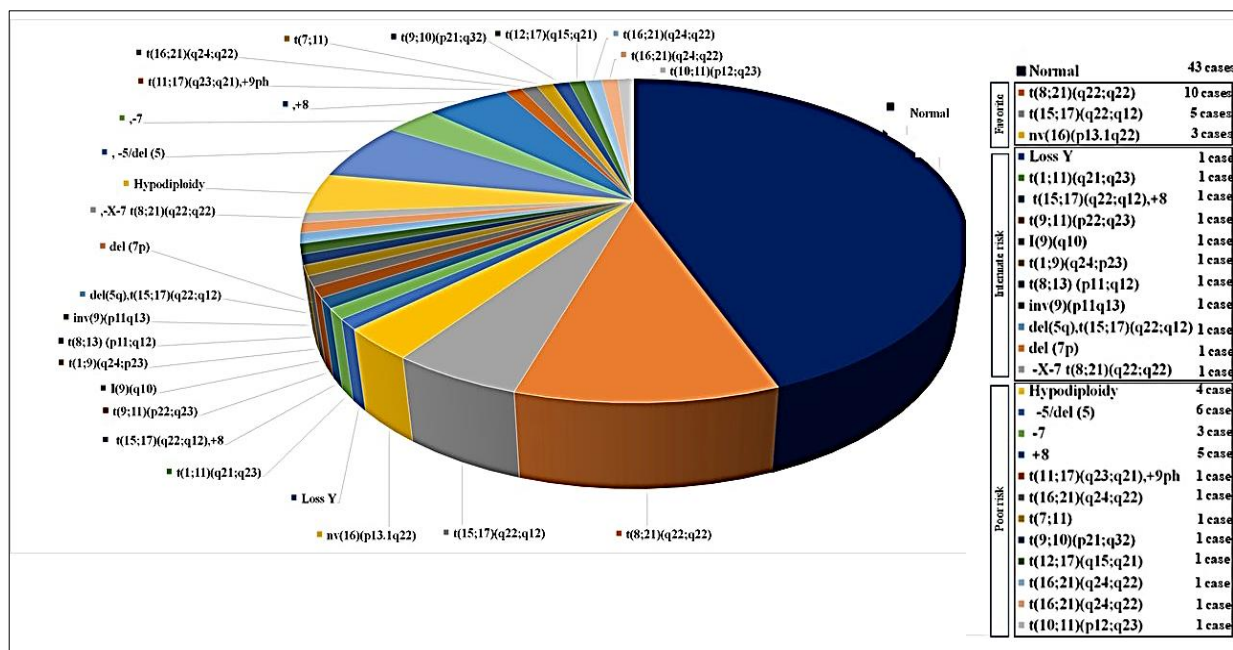


Figure 2: Patient distribution in the current research according to conventional karyotyping data. t: Translocation, ph: polymorphism heterochromatin, Inv: Inversion, I: Isochromosome.

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 | | | | | P value |
|---|-----------------------------------|------------|------------|------------|------------|---------|
| | Normal | Favorite | Intermate | Poor | Total | |
| Total (%) | 43 (43.9%) | 18 (18.4%) | 11 (11.2%) | 26 (26.5%) | 98 (100%) | |
| Gender | | | | | | 0.487 |
| Male | 18 | 10 | 7 | 11 | 46 (46.9%) | |
| Female | 25 | 8 | 4 | 15 | 52 (53.1%) | |
| Age, Year | | | | | | 0.087 |
| 18> | 12 | 4 | 7 | 7 | 30 (30.6%) | |
| 18≤ | 31 | 14 | 4 | 19 | 68 (69.4%) | |
| AML subtypes according to FAB classification | | | | | | 0.000 |
| 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 | | | | | | 0.020 |
| 450> | 17 | 9 | 1 | 4 | 31 (31.6%) | |
| 450< | 26 | 9 | 10 | 22 | 67 (68.4%) | |
| Bone marrow blast (%) | | | | | | 0.022 |
| 50% > | 12 | 10 | 1 | 5 | 28 (28.6%) | |
| 50% < | 31 | 8 | 10 | 21 | 70 (71.4%) | |
| WBC (X 10⁹/L) | | | | | | 0.015 |
| 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 $>50 \times 10^9/L$ (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 | |
| Intermate | 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 ($\times 10^9/L$) | | | | 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.

3.2 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 $\times 10^9/L$, 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 $\times 10^9/L$ (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 \pm 14.56) but it was around (15.80 \pm 14.33) for patients with the poor conventional karyotyping risk, figure 3. 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 \pm SD | P value |
|---|------------|--|---------|
| Gender | | | 0.341 |
| Male | 46 (46.9%) | 22.57 \pm 13.63 | |
| Female | 52 (53.1%) | 19.50 \pm 15.24 | |
| Age, Year | | | 0.353 |
| <18 | 30 (30.6%) | 20.72 \pm 14.43 | |
| >18 | 68 (69.4%) | 21.04 \pm 14.66 | |
| AML subtypes | | | 0.000 |
| M0 | 6 (6.1%) | 11.97 \pm 10.01 | |
| M1 | 10 (10.2%) | 18.16 \pm 15.82 | |
| M2 | 22 (22.4%) | 26.59 \pm 13.20 | |
| M3 | 17 (17.3%) | 23.48 \pm 15.99 | |
| M4 | 8 (8.2%) | 20.66 \pm 6.87 | |
| M5 | 34 (34.7%) | 18.55 \pm 14.63 | |
| M6 | 1 (1.1%) | - | |
| M7 | 0 (0.0%) | - | |
| Conventional karyotyping | | | 0.013 |
| Normal | 43 (43.8%) | 22.40 \pm 14.18 | |
| Favorite | 18 (18.4%) | 24.55 \pm 14.56 | |
| Intermate | 11 (11.2%) | 21.47 \pm 13.64 | |
| Poor | 26 (26.6%) | 15.80 \pm 14.33 | |
| Bone marrow blast (%) | | | 0.000 |
| 50% > | 28 (28.6%) | 31.89 \pm 10.61 | |
| 50% < | 70 (71.4%) | 16.99 \pm 13.79 | |
| LDH U/L | | | 0.000 |
| 450 U/L > | 31 (31.6%) | 30.64 \pm 10.04 | |
| 450 U/L < | 67 (68.4%) | 16.45 \pm 14.18 | |
| WBC ($\times 10^9/L$) | | | 0.000 |
| 50 > | 34 (34.7%) | 31.26 \pm 10.24 | |
| 50 < | 64 (65.3%) | 15.45 \pm 13.55 | |

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.

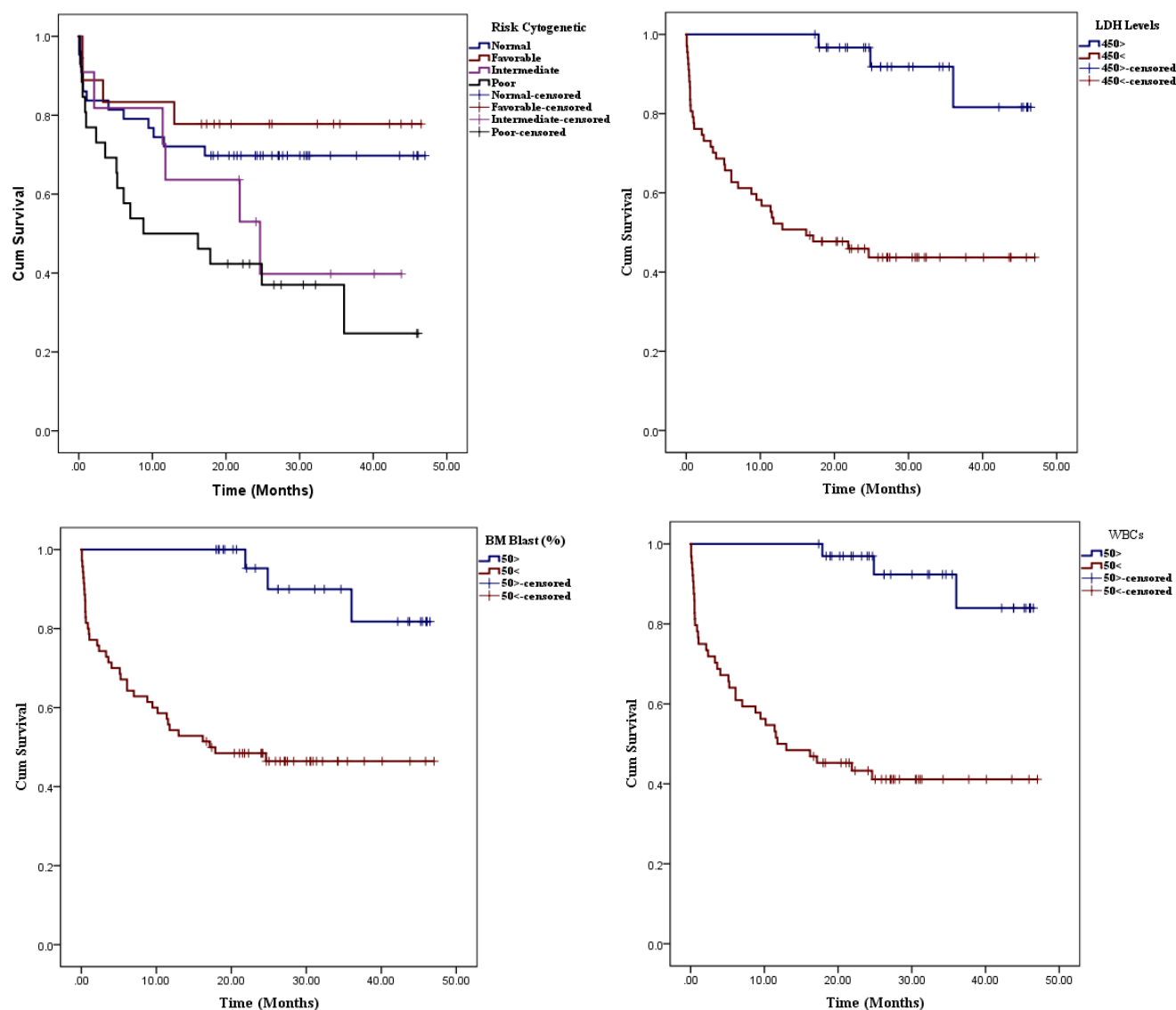


Figure 3: Relation between overall survival times and conventional karyotyping results (A), LDH concentration (B), Bone Marrow blast percentage (C), and white blood cells (D).

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 $\times 10^9/L$ (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 $\times 10^9$ (50> versus 50<) | 0.000 | 0.098 | (0.030-0.320) |

3.3 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.

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 karyotyping | 0.660 | 0.007 |

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

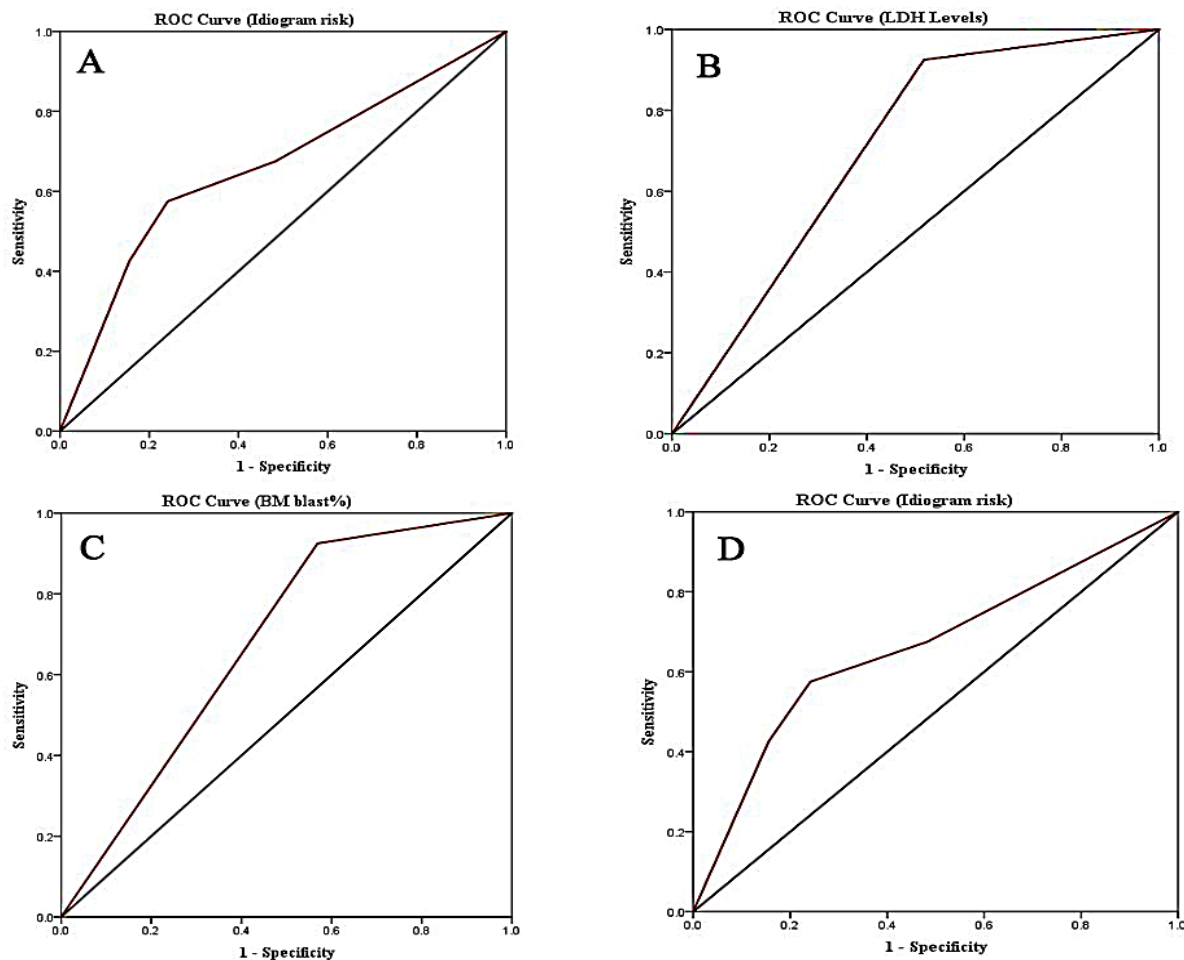


Figure 4: Receiver operating characteristic curves showed sensitivity and specificity for (A) Conventional karyotyping groups risk, (B) Lactate dehydrogenase concentration (IU/L), (C) Bone marrow blasts (%), and (D) White blood cells count $\times 10^9/L$.

4. Discussion

AML is a heterogeneous hematology malignancy, and the mechanism of leukemogenesis is still not fully known [21]. However, the majority of AML patients have acquired genetic abnormalities, including chromosomal abnormalities and somatic mutations, which may play roles in diagnosis and prognosis [1]. Several studies have shown that cytogenetic abnormalities have prognostic value [22,23]. The aim 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 survival [34]. The current study also suggested that the AML-M0 subtype has the worse prognosis in AML subtypes, which is consistent with Walter, R study [35].

Conventional cytogenetics with banding techniques remains the gold standard for chromosomal abnormalities study in AML diagnosis [36]. Nonrandom chromosomal abnormalities had been found by conventional cytogenetics in 60% of adults with AML [7], 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 with results of a previous study [38].

LDH is one of the most important enzymes of the anaerobic metabolic pathway [39]; it is playing an important role as a critical checkpoint of gluconeogenesis and deoxyribonucleic acid (DNA) metabolism [40].

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 effect [9].

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 leukemia [34]. 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 research [43]. Although the causes of hyperleukocytosis are unknown, it has established that malignant blasts may adhere to the vascular endothelium and transmigrate into tissues [44].

Our results showed that an increased BMB count of $<50\%$ has been associated with an adverse prognosis (P value <0.000), consistent with research 16. However, the mechanisms by which leukemic blast mobilization from and homing to the bone marrow occurs remain incompletely defined [23].

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.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship

Each author listed in the manuscript has approved the submission of this version of the manuscript and takes full responsibility for it.

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