

Research Article

## EXPRESSION OF SEVERAL INFLAMMATION-RELATED GENES, IMMUNOPHENOTYPE AND CHROMOSOME ABNORMALITIES IN ACUTE MYELOID LEUKEMIA

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### ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the accumulation of immature myeloid cells in the bone marrow. According to cytogenetic abnormalities, AML patients are stratified into favorable-risk, intermediate-risk, and poor-risk groups; however, differences in inflammatory profiles among these groups remain incompletely understood. This study aimed to investigate the associations between cytogenetic risk categories, clinical characteristics, immunophenotype, and inflammatory expression in AML patients. A total of 63 newly diagnosed AML patients were enrolled. Immunophenotypic profiles were analyzed by flow cytometry, gene expression by quantitative PCR, and cytokine levels by ELISA. The favorable-risk group exhibited significantly lower levels of creatinine, total bilirubin, and indirect bilirubin compared with the intermediate-risk and poor-risk groups. In contrast, nucleated red blood cells and white blood cells were significantly increased in the intermediate-risk group. The AML1-ETO fusion gene was detected in 78.57% of favorable-risk patients and 21.43% of intermediate-risk patients but was absent in the poor-risk group. Immunophenotype analysis indicated that the percentage of CD34<sup>+</sup> cells was higher in the poor-risk group than that in the intermediate-risk and favorable-risk groups, while the numbers of CD56<sup>+</sup> and CD19<sup>+</sup> cells were higher in the favorable-risk group. Moreover, the intermediate-risk and poor-risk patients had enhanced TGF- $\beta$  and CA125 concentrations than the favorable-risk group. Importantly, the nucleated erythrocyte count was enhanced in AML patients with low *CYLD* expression. In conclusion, the results in this study provide an important reference document for identifying the association of clinical features and inflammatory expression among the favorable-risk, intermediate-risk, and poor-risk groups. In particular, several biomarkers, including the low *CYLD* expression, enhanced number of CD34<sup>+</sup> cells, and high TGF- $\beta$  and CA125 levels, are associated with poor outcomes in AML. The results may suggest an important prognosis for *CYLD*-sensitive AML patients.

**Keywords:** A20, acute myeloid leukemia, *CYLD*, CD34, karyotype.

## INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by uncontrolled proliferation of abnormal myeloid precursors (myeloblasts) in the bone marrow and peripheral blood (Cardoso *et al.*, 2024), in which elevated myeloblasts have a negative effect on survival rates. AML affects individuals of any age, but its incidence increases significantly with age (Appelbaum *et al.*, 2006). AML is the most common leukemia in adults and rare in children. The treatment outcome for the elderly population is generally poor, with the overall median survival time at 2.96 months (Sandes *et al.*, 2011). In Southeast Asia, the death cases of AML increased from 6,870 in 1990 to 13,883 in 2021. The age-standardized death rate (ASDR) of AML in Southeast Asia was increased from 2.05% in 1990 to 2.12% in 2021 (Zhou *et al.*, 2024).

AML is associated with genetic and chromosomal abnormalities, with karyotypes classified into favorable-risk, intermediate-risk, and poor-risk groups according to the World Health Organization (WHO) and the European LeukemiaNet (ELN) classification (Lachowicz *et al.*, 2023). Karyotype analysis, observed in about 50–60% of adult AML patients, is a standard diagnostic tool that provides critical prognostic information regarding response to induction therapy, remission duration, and overall survival. (Witherspoon *et al.*, 2001). The favorable-risk group includes patients with normal karyotypes or specific chromosomal abnormalities such as t(8;21), t(15;17), and inv(16) translocations, as well as *NPM1*-mutated AML patients without *FLT3-ITD* (Mrózek, 2008). The intermediate-risk group accounts for around 50% of AML patients and is the most

heterogeneous subgroup of AML. This group includes cases with a normal karyotype as well as those harboring specific abnormalities such as the t(9;11) translocation or *FLT3-ITD* mutations (Kantarjian *et al.*, 2025). Unlike the two groups, AML patients with poor-risk profiles are characterized by the presence of complex and monosomal karyotypes, inv(3), t(3;3), t(6;9), t(9;22), and t(8;16) translocations, *TP53* mutation as well as loss of chromosomes 5 or 7 (Kugler *et al.*, 2024).

In addition to chromosomal abnormalities, the presence of the CD34 marker (Zhi *et al.*, 2010) and the high number of nucleated red blood cells (Girard *et al.*, 2023) are associated with a poor prognosis in AML. They are immature cells and defective in performing their functions, leading to lower survival rates and higher relapse rates in AML patients. CD34 is expressed on leukemic stem cells (LSCs) that drive the development of AML and contribute to resistance to chemotherapy (Jin *et al.*, 2025). Nucleated red blood cells are the precursor cells of the red blood cell lineage and are absent in healthy individuals (Girard *et al.*, 2023).

Tumor necrosis factor- $\alpha$  (TNF $\alpha$ )-induced protein 3 (*TNFAIP3*, *A20*) and tumor suppressor cylindromatosis (*CYLD*) are deubiquitinase genes, which play important roles in negatively regulating nuclear factor (NF)- $\kappa$ B and Janus kinase (JAK)/signal transducer and activator of transcription (STAT)-dependent activation of immune cells (Ha *et al.*, 2025; Huyen *et al.*, 2023). Inactivation of *A20* is associated with its polymorphisms, leukocyte expansion, and poor outcomes in acute lymphoblastic leukemia (Ha *et al.*, 2025). Unlike *A20*, the low *CYLD* expression is linked to older patients and an enhanced level of lactate

dehydrogenase (LDH) in AML (Huyen *et al.*, 2023). *CYLD* participates in promoting cell death in lymphocytic leukemia (Xu *et al.*, 2020). In AML, overexpression of STAT1 and STAT3 is found in almost all AML blasts and their activation leads to immune evasion and the development of AML by modulating cellular physiological processes (Ning *et al.*, 2001; Zhou *et al.*, 2009).

The association of *A20* and *CYLD* with chromosomal abnormalities and immunophenotype in AML is little known, although their effect on the function of leukemic cells is intensively discussed. In this study, we determined the contribution of *A20* and *CYLD*, immunophenotype, and chromosomal abnormalities in AML patients. In addition, their associations with clinical features in Vietnamese AML patients were observed.

## MATERIALS AND METHODS

### Patients and control subjects

Fresh peripheral blood samples were collected from 63 consecutive, untreated patients diagnosed with AML. Diagnoses were established at the National Institute of Hematology - Blood Transfusion, Hanoi, Vietnam, and were based on a comprehensive evaluation including cytomorphology, cytochemistry, immunophenotyping, genetics, and clinical features, in accordance with WHO classification criteria (Arber *et al.*, 2016). Cytogenetic risk is defined by the 2022 ELN risk classification recommendation (Lachowicz *et al.*, 2023).

The control group comprised 50 healthy individuals who were free of any known acute or chronic diseases and were not receiving any medication. All patients and

volunteers provided written informed consent to participate in the study. All personal care and experimental procedures were conducted in accordance with Vietnamese law regarding human welfare and received approval from the Ethical Committee (Approval No. 4-2021/NCHG-HĐĐĐ on 14 January 2021) of the Institute of Genome Research, aligning with the Declaration of Helsinki.

### Karyotyping

Bone marrow specimens were prepared by the short-term culture method and the G-banding method and chromosome staining to analyze the patient's chromosome formula on Giemsa-stained slides and G-banding to detect chromosome abnormalities, as described in detail elsewhere (Sato *et al.*, 2015). This test was performed according to the standard procedure of the Department of Molecular Genetics, National Institute of Hematology - Blood Transfusion.

### Immunostaining and flow cytometry

Samples were analyzed using a BD LSR II flow cytometer (BD Biosciences, San Jose, CA). Gating strategies relied on clearly distinguishable cell populations or, when these were absent, on negative antibody controls. The following monoclonal antibodies were used: CD4, CD7, CD8, CD10, CD13, CD19, CD20, CD23, CD33, CD34, CD38, CD56, CD64, CD79a, CD117, MPO, and HLA-DR. All monoclonal antibodies were purchased from Beckman Coulter (CA, USA) (De Wilde *et al.*, 2017). Antibodies were used at a concentration of 10 µg/mL. After a 60-minute incubation at 4°C, cells were washed twice and resuspended in FACS buffer for subsequent flow cytometry analysis (Huyen *et al.*,

2023). This test is performed according to the standard procedure of the National Institute of Hematology-Blood Transfusion.

### Cytokine quantification

Serum was isolated from the blood samples. Serum and cell supernatant were stored at -20°C until use for ELISA. TGF- $\beta$ , IL-6, TNF $\alpha$ , and CA-125 concentrations were determined by using ELISA kits (Thermo Scientific) according to the manufacturer's protocol.

### RNA extraction and real-time RT-PCR

Total RNA was isolated using the E.Z.N.A. Blood RNA mini kit (Omega, USA) according to the manufacturer's instructions. The hybrid genes RUNX1-RUNX1T1 (AML-ETO), CBFB-MYH11, and mutations in the genes FLT3-ITD, FLT3-TKD, and NPM1 mutA were detected by the real-time RT-PCR method. To determine transcript levels of *A20*, *STAT1*, *STAT3*, *CYLD* and *GAPDH*, quantitative real-time RT-PCR with the LightCycler System (Roche Diagnostics, USA) was applied. The following primers were used: *A20* primers: 5'-TCCTCAGGCTTTGTATTTGA-3' (forward) and 5'-TGTGTATCGGTGCATGGTTTT-3' (reverse); *STAT1* primers: 5'-CCCTTCTGGCTTTGGATTGAA-3' (forward) and 5'-CTTCCCGGGAGCTCTCACTGA-3' (reverse); *STAT3* primers: 5'-GGAGGAGTTGCAGCAAAAAG-3' (forward) and 5'-TGTGTTTGTGCCAGAAATGT-3' (reverse); *CYLD* primers: 5'-TGCCTTCCAACCTCTCGTCTTG-3' (forward) and 5'-AATCCGCTCTTCCCAGTAGG-3' (reverse) and *GAPDH* primers: 5'-GGAGCGAGATCCCTCCAA-3' (forward) and 5'-GGCTGTTGTCA TACTTCTCAT-3' (reverse). PCR reactions

were performed in a final volume of 20  $\mu$ L containing 2  $\mu$ L cDNA, 2.4  $\mu$ L MgCl<sub>2</sub> (3  $\mu$ M), 1  $\mu$ L primer mix (0.5  $\mu$ M of both primers), 2  $\mu$ L cDNA Master SybrGreen I mix (Roche Molecular Biochemicals), and 12.6  $\mu$ L nuclease-free water. The target DNA was amplified during 40 cycles of 95°C for 10 s, 62°C for 10 s, and 72°C for 16 s, each with a temperature transition rate of 20°C/s, a secondary target temperature of 50°C, and a step size of 0.5°C. Melting curve analysis was performed at 95°C, 0 s; 60°C, 10 s; and 95°C, 0 s to determine the melting temperature of primer dimers and the specific PCR products. The ratio between the respective gene and corresponding GAPDH was calculated per sample according to the  $\Delta\Delta$  cycle threshold (Ct) method (Livak and Schmittgen, 2001).

### Statistical analysis

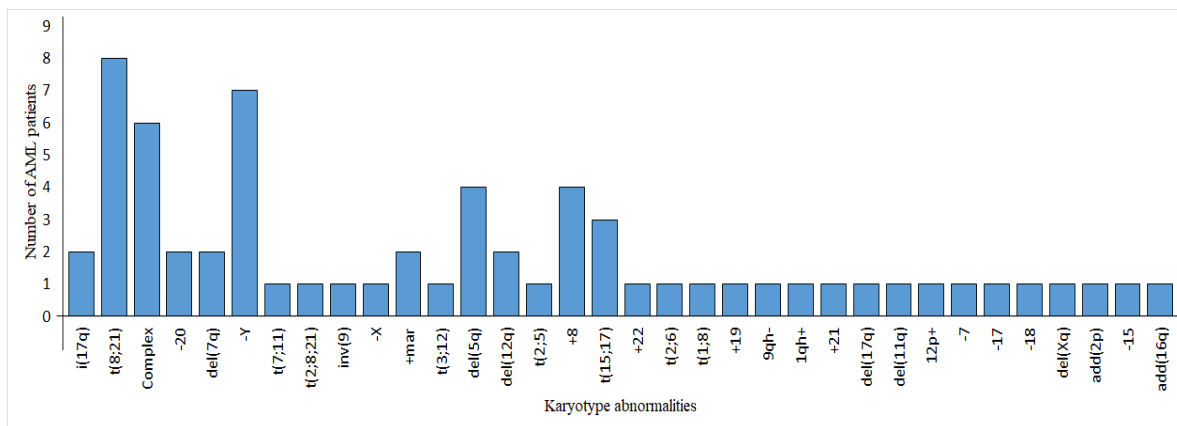
Statistical analysis was performed with SPSS and GraphPad Prism 8.4.3 (San Diego, California, USA). Normally distributed data were presented as mean and standard deviation (mean  $\pm$  SD), and non-normal data were reported as the median with the interquartile range (Q1 and Q3). For comparison of the research groups, the one-way ANOVA test was used for normally distributed data. The Kruskal-Wallis (comparison of multiple groups) and Mann-Whitney U tests (comparison of 2 groups) were used for non-normal data. In all statistical analysis, the level of significance was determined at the level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

Firstly, the analysis of karyotypes from 63 patients diagnosed with AML indicated that 22 (34.92%) patients were of normal karyotype and 41 (65.08%) patients were of

abnormal karyotype. Among the abnormalities observed, the most common abnormality was identified in AML patients with t(8;21), occurring in 8/63 (12.7%) of AML cases. Carriers of Y chromosome deletion were present in 7/63 (11.11%), and those of complex abnormality were in 6/63 (9.52%) (Figure 1). A recent study indicates that a high frequency of chromosomal aberrations is observed in pediatric AML

cases (63.3%) compared to adult AML cases (48.6%) (Vundinti *et al.*, 2023). Chromosomal aberration is associated with progression to AML and plays an important role in the diagnosis and prognosis of hematological malignancies (Witherspoon *et al.*, 2001). In agreement, the percentage of AML cases carrying the t(8;21) is 8% to 20%, which are considered a favorable cytogenetic subgroup (Zhu *et al.*, 2022).



**Figure 1.** Chromosome analysis of AML patients. Add: addition; del: deletion; inv: inversion; t: translocation.

Of the 63 AML patients, 14/63 (22.22%) were grouped into the favorable-risk group, 34/63 (53.96%) were grouped into the intermediate-risk group, and 15/63 (23.82%) were grouped into the poor-risk group based on the ELN risk stratification (Lachowicz *et al.*, 2023) (Table 1). Results of clinical characteristics revealed that the distribution of age in AML patients was different from each other, as the median age of the favorable-risk cases (31.21 years) was lower compared to that of the intermediate-risk (50.53 years) and poor-risk (58.47 years) patients ( $p = 0.004$ ). In agreement, the incidence of AML increases significantly with age (Appelbaum *et al.*, 2006). A recent study

showed that patients under 60 years old have a higher cure rate than those 60 years old and older (Vakiti *et al.*, 2025). In a similar study in AML, the prevalence of complex karyotypes has also been noted to increase with age (Mrózek, 2008). The difference in gender distribution in this study was not statistically significant. Among 63 AML patients, 36/63 (57.14%) were male and 27/63 (42.86%) were female. This is consistent with previous studies showing that AML occurs more frequently in men than in women (Extermann *et al.*, 2023), and the 5-year relative survival rate for women with AML has been reported to be 60.4% compared to 48.8% in men (Ansarian *et al.*, 2025).

**Table 1.** Clinical features of AML groups.

Characteristic	Normal range	AML groups			p value
		Favorable-risk (n = 14), Median (Q1-Q3)	Intermediate-risk (n = 34), Median (Q1-Q3)	Poor-risk (n = 15), Median (Q1-Q3)	
Age (years), mean ± SD		31.2 ± 20.8	50.5 ± 21.3	58.5 ± 15.6	0.004**
Sex, male (n, %)		7 (50)	18 (52.94)	7 (46.67)	0.756
Creatinine (µmol/L)	62-120	61.00 (54.00 - 83.00)	88.50 (69.50 - 99.00)	81.00 (72.00 - 92.00)	0.048*
Total bilirubin (µmol/L)	≤17	7.90 (6.00 - 9.60)	11.100 (7.875 - 14.250)	9.60 (7.00 - 11.70)	0.041*
Indirect bilirubin (µmol/L)	≤12.7	6.10 (4.95 - 8.15)	8.75 (6.40 - 11.250)	7.20 (5.50 - 8.80)	0.021*
Nucleated red blood cell count (G/L)	0	0 (0 - 0.01)	0.04 (0 - 0.20750)	0.020 (0 - 0.040)	0.006**
White blood cell count (G/L)	3.5-10.5	10.13 (4.52 - 24.12)	33.75 (6.58 - 81.38)	6.01 (4.66 - 19.03)	0.015*
Lymphocyte count (G/L)	1.2-3	1.82 (1.15 - 5.305)	3.7 (2.07 - 6.305)	1.74 (1.16 - 2.58)	0.016*

\*p < 0.05 and \*\*p < 0.01 indicate significant differences among the three AML groups.

Moreover, the favorable-risk group with the average levels of creatinine 61.00 µmol/L, total bilirubin 7.90 µmol/L, and indirect bilirubin 6.10 µmol/L was significantly lower than that in both intermediate-risk (88.50, 11.00 and 8.75 µmol/L, respectively) and poor-risk (81.00, 9.60 and 7.20 µmol/L, respectively) groups (Table 1). Bilirubin, which is the final product of heme catabolism, specifically hemoglobin in red blood cells, is conjugated to form water-soluble direct bilirubin to secrete into bile. High serum creatinine levels are a key indicator of renal dysfunction and failure. The indicators are considered potential early

indicators of disease severity (Chen *et al.*, 2025). However, levels of creatinine, total bilirubin and indirect bilirubin in the three groups were not higher than their cutoff values, therefore, the intermediate-risk and poor-risk groups may be linked to a greater risk for liver and kidney impairment. In addition, the high levels of indirect bilirubin are associated with thrombosis in AML (Song *et al.*, 2024).

Next, the number of nucleated red and white blood cells (WBCs) and lymphocytes was higher in the intermediate-risk group (0.04, 33.75 and 3.7 G/L, respectively) compared

with the favorable-risk (0, 10.13 and 1.82 G/L, respectively) and poor-risk (0.02, 6.01 and 1.74 G/L, respectively) groups. The nucleated red cells are immature red blood cell precursors that reside in the bone marrow and are not present in the circulation of healthy adults (Girard *et al.*, 2023), while the WBCs are involved in protecting the body against infection. However, the high number of WBC count in acute leukemia results in stroke, internal bleeding, organ failure, or vision loss (Chennamadhavuni *et al.*, 2025). Older age and high WBC count are risk factors for treatment failure (Ustun *et al.*, 2021). The WBC count elevation in the intermediate-risk group may indicate an aggressive form of leukemia, whereas the low WBC count in the poor-risk group may reflect bone marrow issues and increased susceptibility to infection. In agreement, a recent study indicates that the median of WBC count is reduced in the poor prognosis group (Chen *et al.*, 2023).

In addition, the other clinical characteristics, including urea, glucose, uric acid, direct bilirubin, total protein, albumin, globulin, ferritin, AST, ALT, GGT, LDH, red blood count, hematocrit, reticulocyte ratio, platelet count, blasts and leukocytes (except for

lymphocytes), were found to have no significant differences among the three groups.

In this study, AML with the following gene mutations has been included: *NPM1*, *CEBPA*, *BCR-ABL1*, and *RUNX1*. AML with *NPM1* or *CEBPA* mutations are considered favorable, while AML with *RUNX1* mutations is unfavorable (Carter *et al.*, 2020). Results indicated that 11/14 (78.57%) in the favorable-risk group and 2/34 (5.88%) in the intermediate-risk group were identified with the oncogenic fusion gene *AML1-ETO*, whereas no cases in the poor-risk group were positive with the *AML1-ETO* fusion gene (Figure 2). Differently, the percentages of AML patients positive with *FLT3-ITD*, *FLT3-TKD*, *TP53*, *NPM1*, *RUNX1*, *ASXL1*, and *CEBPA* mutations were similar to each other among the three groups (data not shown). AML carriers of the *AML1-ETO* fusion gene are most commonly younger patients, exhibit high complete remission rates of approximately 85–90% and prolong disease-free survival (Mohamed *et al.*, 2015; Cho *et al.*, 2003). Consistent with this, the poor-risk group in our study had no cases positive for the *AML1-ETO*.

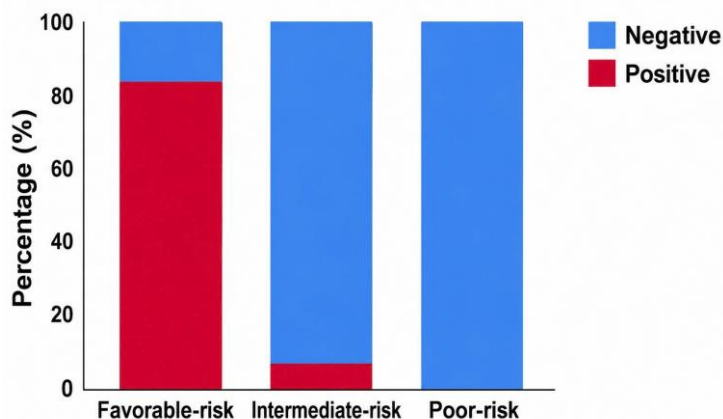


Figure 2. Genetic distribution of AML1-ETO-positive patients.

**Table 2.** Immunophenotype in AML patients.

Immunophenotype	Favorable-risk (n = 14, mean $\pm$ SD)	Intermediate-risk (n = 34, mean $\pm$ SD)	Poor-risk (n = 15, mean $\pm$ SD)	p value
CD4 positive	2.37 $\pm$ 8.22	5.85 $\pm$ 23.3	3.63 $\pm$ 13.6	0.981
CD7 positive	12.14 $\pm$ 28.4	26.23 $\pm$ 36.3	14.07 $\pm$ 21.7	0.353
CD10 positive	0 $\pm$ 0	0 $\pm$ 0	2.62 $\pm$ 9.8	0.2
CD13 positive	41.47 $\pm$ 29.3	28.6 $\pm$ 33.03	49.06 $\pm$ 27.29	0.083
CD19 positive	17.55 $\pm$ 27.54	0 $\pm$ 0	0 $\pm$ 0	<0.001***
CD33 positive	89.19 $\pm$ 11.76	90.9 $\pm$ 13.86	86.5 $\pm$ 16.9	0.116
CD34 positive	74.9 $\pm$ 29.6	39.28 $\pm$ 37.6	66.74 $\pm$ 21.29	0.004**
CD38 positive	2.94 $\pm$ 10.2	0 $\pm$ 0	2.26 $\pm$ 8.47	0.28
CD56 positive	54.19 $\pm$ 44.2	16.38 $\pm$ 29.3	11.27 $\pm$ 29	0.004**
CD64 positive	17.67 $\pm$ 36.7	23.19 $\pm$ 34.8	24.04 $\pm$ 23.3	0.413
CD117 positive	75.77 $\pm$ 15.74	64.68 $\pm$ 32.66	75.51 $\pm$ 17.8	0.816
HLA-DR positive	73.7 $\pm$ 31.3	58.96 $\pm$ 34.9	70.27 $\pm$ 27.34	0.08
MPO positive	95.87 $\pm$ 6.04	74.08 $\pm$ 36.87	82.09 $\pm$ 35.76	0.07

\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  indicate significant differences among the three AML groups.

Immunophenotype analysis demonstrates that leukemic blasts express CD13, CD117, CD23, CD33, CD64, and MPO as myeloid markers; CD4, CD7, and CD8 as T-cell lineage markers; CD10, CD19, CD20, and CD79a as B-cell lineage markers; and CD34, CD38, HLA-DR, and CD56 as nonspecific markers (Table 2) (Yu *et al.*, 2023). In this finding, the percentage of CD34<sup>+</sup> cells was lower in the intermediate-risk (39.28%) than that in the favorable-risk and poor-risk groups (74.9% and 66.74%, respectively). CD34 is highly expressed on multiple cancer stem cells, but not in mature blood and lymphoid cells (Civin *et al.*, 1984; Radu *et al.*, 2023), and plays an important role in blocking cell differentiation (Radu *et al.*, 2023). In agreement, AML patients with a high percentage of CD34<sup>+</sup> cells are associated with a poor prognosis (Zhi *et al.*, 2010). In contrast, the numbers of CD56<sup>+</sup> and CD19<sup>+</sup>

cells were higher in the favorable-risk group (54.19 and 17.55%, respectively) compared to the intermediate-risk (16.38 and 0%, respectively) and poor-risk (11.27 and 0%, respectively) groups. CD56, or NCAM1, which is predominantly expressed in natural killer (NK) cells and related to poor prognosis when expressed in adult AML patients (Liang *et al.*, 2022). CD19 is associated with favorable outcomes in B-cell acute lymphoblastic leukemia (Llaurador *et al.*, 2024), whereas its expression is linked to poor prognosis in others, such as diffuse large B-cell lymphoma and autoimmune diseases (Sermer *et al.*, 2023). In addition, the expression of other markers including CD23, CD8, CD20, and CD79a was negative in all three groups (data not shown). The evidence suggests that AML patients with low expression levels of CD19 and CD56 may contribute to the poor outcome of AML.

**Table 3.** Expression levels of several inflammation-related genes in AML patients.

Characteristic	Healthy controls (n = 50), Median (Q1-Q3)	AML groups			p value
		Favorable-risk (n = 14), Median (Q1-Q3)	Intermediate-risk (n = 34), Median (Q1-Q3)	Poor-risk (n = 15), Median (Q1-Q3)	
A20/GAPDH relative expression	15.12 (0.15 -81.53)	0.6 (0.18 - 1.77)	2.76 (0.79 - 11.34)	1.02 (0.17 - 1.76)	0.013*
CYLD/GAPDH relative expression	9.57 (0.05 -45.36)	0.24 (0 - 0.74)	0.43 (0.23 - 1.47)	0.26 (0.02 - 1.01)	0.095
STAT1/GAPDH relative expression	1.38 (0.11 - 2.09)	0.40 (0.13 - 0.68)	0.51 (0.22 - 1.32)	0.25 (0.06 - 0.52)	0.086
STAT3/GAPDH relative expression	0.78 (0.02 - 1.34)	0.08 (0.02 - 0.77)	0.99 (0.25 - 2.23)	0.14 (0.02 - 0.81)	0.002**

\* $p < 0.05$  and \*\* $p < 0.01$  show significant differences among the three AML groups.

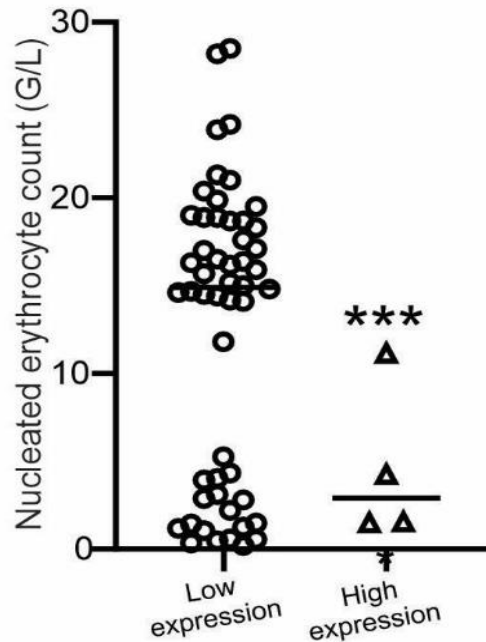
The association between inflammatory expression and accumulation of WBCs and lymphocytes is well-known in AML. Among inflammation-related genes, *A20* and *CYLD* are considered negative regulators of inflammatory response as well as several cellular physiological processes such as cell differentiation, maturation, migration, phagocytosis, and apoptosis (Ning *et al.*, 2001; Zhou *et al.*, 2009). In AML, constitutive activation of STAT1 and STAT3 is detected in almost all AML blasts (Steensma *et al.*, 2006). In this study, the higher *A20* and *STAT3* expression levels were present in the intermediate-risk patients (Table 3). STAT3 contributes to the autonomous proliferation and inhibits the elimination of AML cells from the natural killer cells (Witalisz-Siepracka *et al.*, 2024), which can recognize and kill malignant cells. The high expression levels of *A20* and

*STAT3* in the intermediate-risk group suggested an immunosuppressive response in these patients.

Next, we examine whether an association between *A20* and *CYLD* expression levels with clinical features in AML patients exist. The levels of *A20* and *CYLD* expression in AML patients comprised two (high vs. low) groups based on the median of their values in healthy individuals. Results indicated a significant elevation of nucleated erythrocyte count (4.75-fold) in AML patients with low *CYLD* expression compared with the high *CYLD* expression group (Figure 3). In addition, the association of *A20* and *CYLD* expression and other clinical features in AML patients did not occur (data not shown). In AML, the high number of nucleated red blood cells is associated with a poor prognosis (Girard *et*

*al.*, 2023). Moreover, low *CYLD* expression is linked to inflammatory response in AML (Huyen *et al.*, 2023). Evidences indicated

that AML patients with low *CYLD* expression were sensitive to inflammation and at risk of poor outcomes.



**Figure 3.** Associations of *CYLD* levels with clinical features in AML patients. \*\*\* $p < 0.001$  shows a significant difference from the low *CYLD* expression group.

Finally, we evaluated the changes in inflammatory cytokine and cancer antigen concentrations, including IL-6, TGF- $\beta$ , TNF- $\alpha$ , and CA-125. The inflammatory cytokines are released by immune and abnormal cells, affecting the progression of AML (Reikvam *et al.*, 2015). As shown in Table 4, TGF- $\beta$  and CA125 concentrations were significantly higher in the intermediate-risk (254.82 pg/mL and 5.36 U/mL, respectively) and poor-risk (233.25 pg/mL and 7.73 U/mL, respectively) groups compared with the favorable-risk group (93.25 pg/mL and 5.16 U/mL, respectively). In normal cells,

TGF- $\beta$  inhibits cell proliferation, induces cell differentiation, and apoptosis. In contrast, TGF- $\beta$  promotes cancer cell invasion and contributes to carcinogenesis (Massagué *et al.*, 2000). Moreover, the increased CA125 levels in the intermediate-risk and poor-risk groups may signal the presence of more extensive inflammatory processes or tissue damage in these groups. CA125 is used as a tumor marker, especially in ovarian, endometrial, and peritoneal cancers (Charkhchi *et al.*, 2020). In agreement, the elevated CA125 levels are also observed in leukemia patients (Birgen *et al.*, 2005).

**Table 4.** Cytokine and cancer antigen CA-125 concentrations in AML patients.

Characteristic	AML patients				p value
	Normal range	Favorable-risk (n = 14), Median (Q1-Q3)	Intermediate-risk (n = 34), Median (Q1-Q3)	Poor-risk(n = 15), Median (Q1-Q3)	
IL-6 (pg/mL)	< 7.0	11.89 (5.03 - 16.35)	13.94 (11.41 - 18.72)	17.37 (8.9 - 30.7)	0.095
TGF-β (pg/mL)	670.94 ± 14.4	93.25 (38 - 201.19)	254.82 (144.09 - 454.05)	233.25 (103.93 - 428.45)	0.038*
TNF-α (pg/mL)	< 8.0	0 (0 - 8.56)	0 (0 - 2.17)	5.36 (0 - 17.01)	0.163
CA-125 (U/mL)	< 35.0	5.16 (2.97 - 7.4)	5.36 (0 - 17.01)	7.73 (5 - 11.03)	0.006**

\* $p < 0.05$  and \*\* $p < 0.01$  show significant differences among the three AML groups (ANOVA).

In conclusion, this study reveals that clinical features and inflammatory expression markedly differ among the favorable-risk, intermediate-risk, and poor-risk groups, especially in patients with low *CYLD* expression, an enhanced number of CD34<sup>+</sup> cells, and high TGF-β and CA125 levels are associated with poor outcomes in AML. The results may provide an important prognosis for *CYLD*-sensitive AML patients.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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