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Research Article

EXPRESSION OF IMMUNOPHENOTYPE, INFLAMMATORY RESPONSE AND CHROMOSOMAL ABNORMALITY IN ACUTE LYMPHOID LEUKEMIA

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common childhood malignant hematologic disease characterized by the uncontrolled accumulation of lymphoid progenitor cells within the bone marrow and peripheral blood. According to the World Health Organization (WHO) classification and National Comprehensive Cancer Network (NCCN) guidelines, ALL patients are classified into two main risk groups, including standard-risk and poor-risk groups based on clinical and biological features, including age at diagnosis, white blood cell count, cytogenetic abnormalities, and early treatment response. Inflammation-related genes, such as tumor necrosis factor-α (TNFα)-induced protein 3 (*TNFAIP3, A20*), tumor suppressor cylindromatosis (*CYLD*) and Cezanne genes, are negative regulators of immune cell activation, and cell survival. To this end, 57 patients diagnosed with ALL and 30 healthy subjects were enrolled. Immunophenotype was determined by flow cytometry, gene expression and chromosomal abnormalities by quantitative PCR, and secretion of cytokines by ELISA. As a result, the poor-risk group had age-related higher levels of lactate dehydrogenase (LDH), white blood cell, lymphocytes and neutrophil counts than the standard-risk group. Cytokine analysis revealed that transforming growth factor beta (TGF-β) levels were markedly increased in the poor-risk group. In addition, expression levels of *CYLD, A20*, and *Cezanne* genes were not significantly different between the two groups, although *CYLD* levels tended to be lower in the poor-risk group. Importantly, p210 *BCR-ABL* and p190 *BCR-ABL* fusion transcripts were detected more frequently in poor-risk patients. In conclusion, this study indicates that age-related numbers of malignant lymphoid cells, but not inflammatory expression, were associated with poor outcomes in ALL patients.

Keywords: Acute lymphoblastic leukemia, *BCR-ABL,* malignant lymphoid cells, TGF-β.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood hematologic malignancy characterized by the uncontrolled proliferation and accumulation of lymphoid progenitor cells within the bone

marrow and peripheral blood (Pui and Jeha, 2007). More than 80% of children diagnosed with ALL can be cured with multi-agent treatment regimens, whereas for most adult ALL patients, cure rates are more variable, with a five-year event-free survival around 40% (Chao *et al.*, 2011). ALL with hyperleukocytosis at diagnosis is associated with early morbidity and mortality due to the expression of leukostasis that blocks blood vessels, causing serious, life-threatening issues, including respiratory failure, seizures, bleeding, and kidney failure (Park *et al.*, 2020).

Multiple factors have been considered as important prognostic factors for ALL, including initial white blood cell (WBC) count, age, immunophenotype, cytogenetics and absolute lymphocyte count (Farkas *et al.*, 2017). According to National Comprehensive Cancer Network (NCCN) guidelines, patients with ALL are classified into two main risk groups, including poor-risk and standard-risk groups based on factors like age, WBC count, genetics (e.g., specific gene fusions, hypodiploidy), and response to initial treatment (Brown *et al.*, 2021). The poor-risk group is present with poor-risk cytogenetic abnormalities (e.g., t(9;22), t(4;11)), age >60 years, WBC count >100,000/mm³ at diagnosis and certain genetic changes like Philadelphia chromosome-like (Ph-like) and T-cell ALL subtypes (Brown *et al.*, 2021). In pediatric patients, this group includes those ≥10 years of age or presenting with a WBC ≥50,000/μL at diagnosis (Shah *et al.*, 2024). Differently, standard-risk ALL is absent of all poor-risk cytogenetic abnormalities. This group includes children aged 1 through 9 years with a WBC count of less than 50,000 cells/μL (Shah *et al.*, 2024) and the presence of favorable cytogenetic subgroups, such as high hyperdiploidy or the translocation t(12;21) (ETV6-RUNX1) (Brown *et al.*, 2021).

Genetic abnormalities frequently associated with ALL include breakpoint cluster region (*BCR*) from chromosome 22 and the Abelson tyrosine kinase (*ABL*) gene from chromosome 9 (*BCR-ABL)*, *IKZF1, PAX5, CDKN2A/B, EBF1, RB1, TP53, JAK1/2, IL7R, CRLF2, FLT3, KRAS, NRAS* and *KMT2A* rearrangement (Kantarjian and Jabbour, 2025). Among them, *BCR-ABL* is a chimeric oncogene generated by translocation of sequences from the *c-ABL* gene on chromosome 9 into the *BCR* gene on chromosome 22. Transcription of the *BCR/ABL* fusion gene results in a hybrid mRNA that is translated into a 210 kDa or 190 kDa protein (Baccarani *et al.*, 2019). The presence of *BCR-ABL* variants, including p190 and p210, is frequently associated with more aggressive disease course, treatment resistance, and poor prognosis (Junmei *et al.*, 2015). Overexpression of the BCR/ABL protein increases proliferation of $CD34^+$ myeloid haematopoietic progenitor cells (Primo *et al.*, 2006), which contribute to poor prognosis of leukemia patients (Modi *et al.*, 2007). The p210 BCR-ABL is also known to inhibit cell apoptosis and is used to determine residual disease in chronic myeloid leukemia patients (Adnan-Awad *et al.*, 2021). The 210-kDa chimeric BCR-ABL protein promotes tyrosine kinase activity, leading to abnormal expression of other signaling molecules and cellular physiological processes in patients with leukemia (Ren, 2005; Amarante-Mendes *et al.*, 2022). Unlike p210 BCR-ABL, the p190 BCR-ABL plays a critical role in the pathogenesis of Philadelphia-positive (Ph+) ALL patients (Fizzotti *et al.*, 1994). In addition, a significant decrease of

platelet count is associated with a significant increase of serum lactate dehydrogenase (LDH) levels and WBC count in ALL (Hafiz and Mannan, 2007).

Immunophenotypic analysis of ALL reveals that elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-6 have been associated with poor outcomes in ALL, whereas a decrease in transforming growth factor beta (TGF-β) levels contributes to disease progression (Radwan *et al.*, 2024). TNF-α and IL-6 are pro-inflammatory cytokines involved in chronic inflammation in hematologic malignancy development and progression, in which IL-6 promotes the growth of blast cells (Naji *et al.*, 2024). Differently, TGF-β has a suppressive effect on cell growth, differentiation and apoptosis (Lin *et al.*, 2005) and promotes immune evasion of blast cells (Bakhtiyari *et al.*, 2023). For multivariate analysis in ALL by flow cytometry, leukemic blasts expressing CD13, CD117, CD23, CD33, CD64 and MPO are considered as myeloid markers; CD3, 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 (Yu *et al.*, 2023).

Our recent study indicates associations among expression levels/polymorphisms of tumor necrosis factor-α (TNFα)-induced protein 3 (*TNFAIP3, A20*), tumor suppressor cylindromatosis (*CYLD*) and Cezanne genes, clinical features, immunophenotype and inflammatory expression in leukemia and lymphoma patients (Ha *et al.*, 2025; Huyen *et al.*, 2023). These genes have been considered as negative regulators of nuclear factor (NF)-κB- and janus kinase/signal transducers and activators of transcription (JAK/STAT) dependent inflammatory response (Ha *et al.*, 2025; Huyen *et al.*, 2023). Inactivation of *A20* is associated with poor outcomes in ALL (Ha *et al.*, 2025), while *CYLD* participates in promoting the cell death in lymphocytic leukemia (Xu *et al.*, 2020) as well as macrophage phagocytosis of leukemic cells in acute myeloid leukemia (Huyen *et al.*, 2023).

In this study, we performed experiments to determine the associations of *A20*, *CYLD* and *Cezanne* expression levels, immunophenotype, inflammatory response and chromosomal abnormalities between the poor-risk and standard-risk groups in 57 ALL patients. Expression levels of *A20*, *CYLD* and *Cezanne* in 57 patients with ALL and 30 healthy subjects were measured by quantitative real-time RT-qPCR to determine the association of the *A20* gene with clinical features in AML. Immunophenotyping of leukemic cells was also examined by flow cytometry.

MATERIALS AND METHODS

Patients and control subjects

Fresh peripheral blood samples were collected from 57 consecutive, untreated patients diagnosed with ALL and 30 healthy subjects. 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 World Health Organization (WHO) classification criteria (Arber *et al.*, 2016). Cytogenetic risk is defined by the 2024 National Comprehensive Cancer Network (NCCN Guidelines) risk classification recommendation (Shah *et al.*, 2024).

Control group participants 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. 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: CD3, CD4, CD7, CD8, CD10, CD13, CD19, CD20, CD33, CD34, CD38, CD45, CD56, CD117, 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-β, IL-6 and TNFα concentrations were determined by using the Human TGF beta-1 ELISA kit, the Human IL-6 ELISA Kit, the Human IL-35 ELISA Kit and the Human TNF alpha ELISA Kit (Invitrogen - Thermo Scientific, USA) according to the manufacturer's protocols.

RNA extraction and RT-PCR

Total RNA was isolated using the E.Z.N.A Blood RNA mini kit (Omega Bio-Tek, USA) and cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The presence of fusion oncogenes, including p190 *BCR-ABL,* p210 *BCR-ABL, MLL-AF4, TEL-AML1* (*ETV6-RUNX1*), and *E2A-PBX1* (*TCF3-PBX1*) was assessed by the reverse transcription polymerase chain reaction (RT-PCR) method. The primer sequences used for detection of fusion genes were as follows: BCR-ABL-F (5′-GATGCTG ACCAACTCGTGTGTG-3′) and BCR-ABL-R (5′-TGGCCACAAAATCATACA GTGC-3′); nested PCR primers B-A 1stF (5′-CAAGGCTACGGAGAGGC-3′), B-A 1stR (5′-ATGGTACCAGGAGTGTTTCT C-3′), B-A 2ndF (5′-GGAGCTGCAGATG CTGACC-3′), B-A 2ndR (5′-TTCCTTGGA GTTCCAACGAGC-3′), B-Am 2ndF (5′-CAGTGCCATAAGCGGCACC-3′), and B-

Am 2ndR (5′-TTCCTTGGAGTTCCAACG AGC-3′). Detection of *TEL-AML1* was performed using TEL-C (5′-AAGCCCATC AACCTCTCTCATC-3′), and AML1-D (5′-TGGAAGGCGGCGTGAAGC-3′). *MLL-AF4* was identified using AF4-D (5′-CGTTC CTTGCTGAGAATTTG-3′) and MLL-E5 (5′-AAGCCCGTCGAGGAAAAG-3′). *E2A-PBX1* was detected using E2A-A (5′-CACCAGCCTCATGCACAAC-3′), and PEX1-B (5′-TCGCAGGAGATTCATCAC G-3′). PCR products were separated by electrophoresis on 2% agarose gels prepared in 0.5× TBE buffer and visualized using a Gel Doc imaging system (Bio-Rad). The presence of fusion transcripts was confirmed based on the expected amplicon sizes.

PCR reactions were performed in a total volume of 20 µL containing 1 µL of cDNA template, 1 µL of Taq DNA polymerase, 1 µL of gene-specific primer pair, and the appropriate reaction buffer provided by the manufacturer. Each fusion gene was amplified using its corresponding primer set. For targets requiring enhanced sensitivity, a nested PCR strategy was employed, in which 1 µL of the first-round PCR product was used as the template for the second-round amplification. Annealing temperatures and cycling conditions were optimized individually for each primer pair. The tests were performed according to the standard procedure of the Department of Molecular Genetics, National Institute of Hematology - Blood Transfusion.

Real-time RT-qPCR

The expression levels of *A20, CYLD, Cezanne* and the internal reference gene *GAPDH* were quantified by real-time quantitative PCR (RT-qPCR) using the LightCycler system (Roche Diagnostics). Gene-specific primers were designed as follows: *A20* (forward: 5′-TCCTCAGGCTT TGTATTTGA-3′; reverse: 5′-TGTGTATC GGTGCATGGTTTT-3′), *Cezanne* (forward: 5′-ACAATGTCCGATTGGCCAGT-3′; reverse: 5′-ACAGTGGGATCCACTTCAC ATTC-3′), *CYLD* (forward: 5′-TGCCTTC CAACTCTCGTCTTG-3′; reverse: 5′-AAT CCGCTCTTCCCAGTAGG-3′), and *GAPDH* (forward: 5′-GGAGCGAGATCCCTCCAA A-3′; reverse: 5′-GGCTGTTGTCATACTT CTCAT-3′). Each PCR reaction was carried out in a total volume of 20 µL, containing 2 µL of cDNA template, 2.4 µL of $MgCl_2$ (3 µM), 1 µL of primer mixture (0.5 µM each), 2 µL of SYBR Green I Master Mix (Roche Molecular Biochemicals, USA), and nuclease-free water to volume. Amplification was performed under the following cycling conditions: initial denaturation at 95°C for 10 s, followed by 40 cycles of 95°C for 10 s, 62°C for 10 s, and 72°C for 16 s. Following amplification, melting curve analysis was conducted to verify product specificity and exclude non-specific amplification or primer–dimer formation. Samples were briefly heated to 95°C, cooled to 60°C, and then subjected to a gradual temperature increase from 60°C to 95°C at a rate of 0.1°C/s, with continuous fluorescence monitoring to determine the melting temperature of each amplicon.

All reactions were performed in triplicate, and *GAPDH* was consistently used as the reference gene. Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method as described elsewhere (Livak and Schmittgen, 2001). Briefly, Ct values of target genes were normalized to *GAPDH* to obtain ΔCt values, which were then compared with the mean ΔCt of healthy control samples to generate ΔΔCt values. Expression data were subsequently log_2-transformed for statistical analysis.

Differences among ALL subgroups were evaluated using one-way ANOVA.

Statistical analysis

Differences were tested for significance using the Mann–Whitney U and Kruskal–Wallis tests. In all statistical analyses, the level of significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Clinical features of the standard-risk and poor-risk groups in ALL patients

The poor-risk prognostic variables of ALL include the WBC count, peripheral blast cell count, hemoglobin level, platelet count and age at diagnosis (Donadieu *et al.*, 2000). In the present study, of the total 57 cases included in the study, standard-risk cytogenetics comprised 36 (63.16%) cases, and poor-risk 21 (36.84%) cases (Table 1). The mean age of patients in the poor-risk group (40.0 ± 18.0 years) was significantly higher than that in the standard-risk group (28.8 ± 20.6 years; $p = 0.044$). It is consistent with a previous report, indicating that higher age is an unfavorable prognostic factor for ALL patients (Pui and Jeha, 2007). In contrast, blood glucose levels were significantly lower in the poor-risk group (5.3 mmol/L) compared with the standard-risk group (6.4 mmol/L; $p = 0.044$), suggesting that the standard-risk group was at an increased risk of impaired glucose metabolism. Leukemic cells are known to consume glucose at a much higher rate than normal cells (Ye *et al.*, 2018). Differently, a recent study indicated that ALL patients with lower age group develop hypoglycemia (Panigrahi *et al.*, 2016).

Next, in agreement with a recent study (Hafiz and Mannan, 2007), the serum LDH concentration and WBC count are significantly higher, whereas the levels of hemoglobin and hemetocrit as well as platelet count are lower in ALL cases than in healthy individuals. A previous study showed that age and the WBC count are the most significant prognostic variables in ALL, whereas the hemoglobin level and the platelet count had a lower prognostic value (Donadieu *et al.*, 2000). Moreover, LDH concentration was significantly higher in the poor-risk group (2230 U/L) compared with the standard-risk group (1078 U/L; $p = 0.013$) (Table 1), suggesting enhanced cellular breakdown and the high glycolytic activity typical of rapidly proliferating malignant cells (Jedlička *et al.*, 2022). A significant decrease in platelet count is in relation to a significant increase in serum LDH level and WBC count in ALL (Hafiz and Mannan, 2007). Similarly, there is a positive relationship in high-risk acute myeloid leukemia between elevated LDH levels and leukocytosis (Jedlička *et al.*, 2022).

At diagnosis, the mean WBC count was significantly higher in the poor-risk group (65.58 G/L) compared with the standard-risk group (10.1 G/L; $p = 0.003$). ALL with hyperleukocytosis at diagnosis is associated with early morbidity and mortality due to the presence of end-organ injury and blocking small blood vessels (Park *et al.*, 2020). Similar to the WBC count, the absolute neutrophil and lymphocyte counts were also markedly elevated in the poor-risk group. An increase in absolute neutrophil counts in ALL is a risk factor for relapse due to effects from inflammatory responses or blast infiltration (Mosher *et al.*, 2022). Absolute lymphocyte count has been indicated as a prognostic factor of survival in ALL;

however, low lymphocyte count might result from sepsis (Farkas *et al.*, 2017).

In addition, no significant associations were found between the two ALL risk groups, including liver (AST, ALT, GGT, and bilirubin) and renal (urea and creatinine) function, as well as other basic hematological indices (Table 1).

Table 1. Hematological parameters in ALL patients.

Characteristic	Normal range	ALL patients		*p*-value
		Standard-risk (n = 36)	Poor-risk (n = 21)	
Age (years)		28.8 ± 20.6	40.0 ± 18.0	0.044*
Urea (mmol/L)	2.5 - 7.5	5.4 (4.03 - 6.7)	5.3 (4.0 - 7.05)	0.862
Glucose (mmol/L)	3.9 - 6.4	6.4 (5.3 - 7.7)	5.3 (5.0 - 6.3)	0.044*
Creatinine (µmol/L)	62-120	80.5 (63.75 - 103)	88 (67.5 - 110)	0.673
Uric acid (µmol/L)	420 (M)/360 (F)	456.5 (347.25 - 576)	514 (417.5 - 586.5)	0.23
Total bilirubin (µmol/L)	≤ 17	11.45 (8.78 - 22.13)	9.9 (7.5 - 17.35)	0.669
Direct bilirubin (µmol/L)	≤ 4.3	2.85 (1.3 - 6.68)	1.9 (1.55 - 3.95)	0.662
Indirect bilirubin (µmol/L)	≤ 12.7	9.45 (7.33 - 16.5)	8.2 (6.2 - 12.95)	0.461
Total protein (g/L)	65 - 82	74.45 (68.2 - 78.28)	70.9 (64.3 -78.35)	0.388
Albumin (g/L)	35 - 50	39.5 (35.25 – 42.0)	39.9 (32.35 - 41.75)	0.588
Globulin (g/L)	24 - 38	31.2 (28.75 - 36.05)	32 (27.6 -37.25)	0.638
Ferritin (µg/L)	30 - 300	620 (336.2 - 811.1)	725.75 (436.73 - 1397.58)	0.178
AST (GOT) (U/L)	≤ 37	41.0 (22.0 – 64.0)	58.5 (38.0 - 101.25)	0.113
ALT (GPT) (U/L)	≤ 40	34.0 (20.0 – 81.0)	41.5 (22.25 - 64.75)	0.63
GGT (UI/L)	≤ 60	82.4 (67.35 - 250.8)	116.55 (52.05 - 337.73)	0.938
Erythrocyte count (T/L)	3.9 - 5.03	3.42 (2.91 - 3.97)	3.62 (3.1 - 4.4)	0.447
Hemoglobin (g/L)	120 - 155	94.0 (73.5 - 112.75)	104.0 (86 - 119.5)	0.35
Hematocrit (%)	37 - 43	27.5 (23.6 - 34.85)	32.4 (27.05 - 37.45)	0.23

Nucleated erythrocyte count (G/L)	0.0	0.02 (0 - 0.26)	0.09 (0 - 0.28)	0.168
Reticulocytes (%)	0.5 - 1.5	0.95 (0.48 - 1.39)	1.21 (0.82 - 1.83)	0.15
LDH (U/L)	230 - 460	1078 (477 - 2718)	2230 (1538.25 - 4824)	0.013*
Platelet count (G/L)	150 - 450	55.0 (22.0 – 95.0)	38.0 (19.0 - 201.5)	0.888
WBC count (G/L)	3.5 - 10.5	10.1 (2.45 - 48.66)	65.58 (22.27 - 127.41)	0.003**
Neutrophil count (G/L)	2.8 - 5.5	1.84 (0.54 - 5.82)	4.39 (2.55 - 10.24)	0.004**
Eosinophil count (G/L)	0.16 - 0.8	0.03 (0.0 - 0.25)	0.0 (0.0 - 0.19)	0.422
Basophil count (G/L)	0.01 - 0.12	0.02 (0.0 - 0.13)	0.0 (0.0 - 0.04)	0.191
Monocyte count (G/L)	0.05 - 0.3	0.26 (0.05 - 0.81)	0.72 (0.0 - 2.15)	0.388
Lymphocyte count (G/L)	1.2 - 3.0	2.44 (1.31 - 7.14)	6.74 (3.24 - 14.51)	0.001**

*ALT: alanine aminotransferase; AST: aspartate transaminase; GGT: gamma glutamyl transferase; LDH: lactate dehydrogenase; and WBC: white blood cell. *p < 0.05 and **p < 0.01 show significant differences among the two ALL groups (ANOVA).*

Inflammatory expression in patients with ALL

For determination of immunophenotype in ALL, we analyzed the expression of CD13, CD117 and CD33 as myeloid markers; CD3, 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 (Yu *et al.*, 2023). Among them, $CD19^+$ and $CD10^+$ cells, which are precursor-B cells and associated with poor prognosis (Wiemels, 2012). In agreement, the poor-risk group had the higher percentages of CD19-positive and CD10-positive cells, 95.1% and 92.8%, respectively, as compared to the standard-risk group (87.9% and 79.4%, respectively). Similar to $CD19^+$ and $CD10^+$ cells, the percentage of blast cells was higher in the poor-risk group than in the standard-risk group (Table 2). A previous study indicated that the numbers of CD10 and CD19-positive cells, which are lymphoid-related markers related to the expression of blast cells and important for the diagnosis and the detection of minimal residual disease of ALL (Rego *et al.*, 1999).

Table 2. Inflammatory expression in ALL patients.

Characteristic	Healthy controls	ALL patients		*p-value*
		Standard-risk (n = 36), Median (Q1-Q3)	Poor-risk (n = 21), Median (Q1-Q3)	
CD3 positive cells (%)	55-75	2.69 (0.0 - 16.15)	1.19 (0.0 - 5.29)	0.687
CD4 positive cells (%)	30-60	10.24 (1.27 -29.3	4.57 (1.8 - 20.4)	0.447
CD7 positive cells (%)	55-75	21.7 (11.78 - 37.4	12.87 (3.14 - 30.8	0.375
CD8 positive cells (%)	15-35	9.72 (0.35 - 25.6)	6.31 (1.57 - 19.8)	0.608
CD10 positive cells (%)	< 5.0	79.4 (0.0 - 96.55)	92.8 (83.28 - 97.03)	0.05*
CD13 positive cells (%)	50-70	6.61 (0.0 - 18.2)	0.0	0.112
CD19 positive cells (%)	5-20	87.9 (0.25 - 97.08)	95.1 (92.63 - 98.05)	0.035*
CD20 positive cells (%)	5-20	0.0 (0.0 - 39.1)	0.0 (0.0 - 27.25)	0.461
CD33 positive cells (%)	40-60	14.79 (0.0 - 29.39	18.94 (0.0 - 30.27	0.62
CD34 positive cells (%)	< 1.0	11.1 (0.0 - 95.1)	57.1 (5.0 - 93.15)	0.417
CD38 positive cells (%)	20-40	2.63 (0.0 - 15.6)	4.23 (0.0 - 18.9)	0.735
CD45 positive cells (%)	95-100	91 (76.78 - 98.3)	91.9 (53.1 - 98.35)	0.317
CD56 positive cells (%)	5-20	2.1 (0.0 - 12.2)	3.64 (0.0 - 16.2)	0.691
CD79a positive cells (%)	5-20	89.45 (7.53 - 96.78)	93.1 (86.0 - 97.58)	0.068
CD117 positive cells (%)	< 1-2	16.12 (7.12 - 31.16)	6.97 (1.56 - 23.26)	0.548
HLA DR positive cells (%)	10-25	63.45 (0.0 - 86.8)	65.47 (0.0 - 93.15)	0.921
Blast cells (%)	<1.0	78 (55.5 - 90.1)	80.5 (76.38 - 92.38)	0.043*
IL6 (pg/mL)	< 7.0	7.66 (4.01 - 14.9)	8.35 (2.78 - 19.26)	0.974
TNF-α (pg/mL)	< 8.0	0.93 (0.7 - 2.7)	0.86 (0.76 - 0.93)	0.636
TGF-β (pg/mL)	2.000 - 4.000	192.37 (85.67 - 369.85)	328.38 (235.43 - 523.79)	0.02*
IL-35 (U/mL)	< 20	1.66 (0 - 35.24)	0.62 (0 - 16.23)	0.535
Relative expression of *CYLD/GAPDH*	9.57 ± 43.36	5.6 (0.4 - 7.21)	0.44 (0.23 - 2.38)	0.115
Relative expression of *A20/GAPDH*	15.32 ± 78.33	3.12 (0.69 - 11.96)	2.03 (0.43 - 9.99)	0.653
Relative expression of *Cezanne/GAPDH*	0.46 ± 1.4	9.61 (4.75 - 11.09)	14.36 (3.11 - 20.13)	0.261

**p < 0.05 show significant differences among the two ALL groups (ANOVA).*

Next, inflammatory cytokines such as IL-6 and TNF-α may contribute to maintaining the malignant phenotype of leukemic cells (Hunter and Jones, 2015; Balkwill, 2009). In contrast, immunosuppressive cytokines such as TGF-β and IL-35 are maintaining immune tolerance or driving pathology (Li *et al.*, 2006; Collison *et al.*, 2012). Our recent study indicated that levels of IL-6, IL-35 and TNF-α were elevated, while TGF-β concentration was reduced in ALL patients compared with healthy controls (Ha *et al.*, 2025). However, no significant differences in the levels of IL-6, IL-35 and TNF-α were found between the two risk groups. Importantly, TGF-β levels were significantly elevated in the poor-risk group, as poor-risk patients had significantly higher TGF-β concentrations (328.38 pg/mL) compared with the standard-risk group (192.37 pg/mL; $p = 0.02$). TGF-β contributes to immune evasion by inhibiting the proliferation of effector T and NK cells (Bakhtiyari *et al.*, 2023). The significantly higher levels of TGF-β observed in the poor-risk group further support its role as a key immunosuppressive cytokine that facilitates the survival and expansion of leukemic cells (Shi *et al.*, 2022). However, the levels of TGF-β are significantly reduced in ALL patients at the time of diagnosis (Schober *et al.*, 2023).

In addition, analyses of gene expression levels of *CYLD, A20,* and *Cezanne,* which are negative regulators of inflammatory response and diverse cellular processes such as cell differentiation, maturation, cytokine secretion, migration, phagocytosis and apoptosis were examined (Espinosa *et al.*, 2010; Gu *et al.*, 2024). Results revealed that no statistically significant differences were seen between the poor-risk and standard-risk groups for their expression levels ($p > 0.05$). *CYLD* expression was lower in the poor-risk group (0.44) compared with the standard-risk group (5.6), whereas *Cezanne* expression showed a trend toward higher levels in the poor-risk group (14.36) compared with the standard-risk group (9.61), although the differences did not reach statistical significance. Activation of *CYLD* induces macrophage phagocytosis of leukemic cells in acute myeloid leukemia (Huyen *et al.*, 2023). Therefore, expression levels of *CYLD, A20,* and *Cezanne* may not be risk factors in ALL. Evidences indicated that the association between the poor-risk and standard-risk groups in ALL may be the presence of malignant lymphoid markers, rather than inflammatory expression.

Gene mutation in ALL patients

Analysis of genetic alterations in ALL by the presence of p190 *BCR-ABL*, p210 *BCR-ABL*, *MLL-AF4, TEL-AML1 (ETV6-RUNX1)*, and *E2A-PBX1 (TCF3-PBX1)* mutations was performed. Results revealed a clear association between the distribution of *BCR-ABL* mutations and the two prognostic groups in 57 ALL patients. In the standard-risk group, no cases of p210 *BCR-ABL* were detected, whereas 14.04% of patients in the poor-risk group were positive for p210, a statistically significant difference ($p = 0.021$). Similarly, the frequency of p190 *BCR-ABL* positivity was 14.04% in the standard-risk group and increased to 38.6% in the poor-risk group, also reaching statistical significance ($p = 0.037$) (Figure 1).

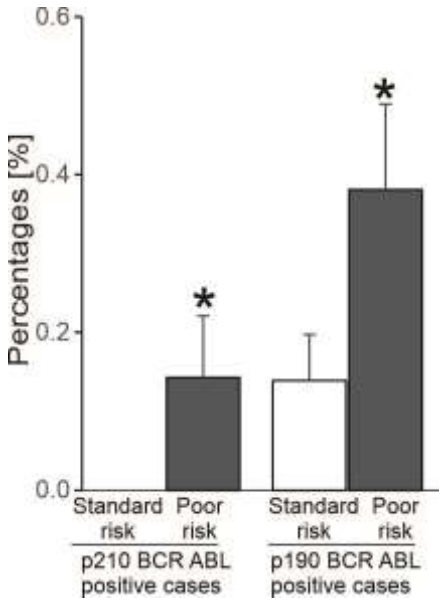

Figure 1. The expression of *BCR-ABL* mutations in the standard risk (n = 36) and poor risk (n = 21) groups of ALL patients. *p < 0.05 show significant differences among the two ALL groups (ANOVA).

These findings indicate that the presence of *BCR-ABL* mutations, particularly the p210 and p190 isoforms, was strongly associated with an unfavorable prognosis in ALL. Our results are consistent with multiple international studies identifying *BCR-ABL* as a high-risk genetic factor, contributing to higher relapse rates, reduced treatment responses, and poorer survival outcomes (Junmei *et al.*, 2015). This finding is consistent with the well-established pathogenic mechanism of *BCR-ABL*, an aberrantly activated tyrosine kinase that drives lymphoblast proliferation and inhibits apoptosis through key signaling pathways such as RAS/MAPK, PI3K/AKT, and JAK/STAT (Ren, 2005; Amarante-Mendes *et al.*, 2022). Sustained activation of these pathways not only leads to an increased number of circulating blasts but also promotes metabolic dysregulation in leukemic cells, contributing to the elevated LDH levels observed in the poor-risk group in our study.

Beyond its direct impact on cellular proliferation, *BCR-ABL* has also been shown to modulate immune responses by influencing the cytokine network (Junmei *et al.*, 2015). Previous studies demonstrated that *BCR-ABL* signaling can activate STAT3 and NF-κB, leading to increased secretion of IL-6 and TNF-α, thereby sustaining a chronic inflammatory *milieu* that favors blast survival (Meyer and Levine, 2014; Brück *et al.*, 2020). Our findings are consistent with this observation, as IL-6 and TNF-α levels were elevated in ALL patients compared to healthy controls. Moreover, *BCR-ABL* mutations may indirectly promote the upregulation of immunosuppressive cytokines such as TGF-β, which contribute to the depletion of normal lymphocyte populations and facilitate immune evasion by malignant cells (Hu and Ivashkiv, 2009). Differently, the p210 *BCR-ABL* gene is observed in most Ph-positive CML patients, while the breakpoint in p190 *BCR-ABL* is

more frequently found in Ph-positive ALL (Junmei *et al.*, 2015).

In conclusion, this study reveals that age-related numbers of CD19 and CD10 positive cells and blast cells, rather than inflammatory expression were linked to poor outcomes in ALL patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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