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Original article

A RETROSPECTIVE ANALYSIS OF PATIENTS DIAGNOSED WITH ACUTE LYMPHOBLASTIC LEUKEMIA OVER FOUR CONSECUTIVE YEARS AT THE UNIVERSITY CLINIC FOR HEMATOLOGY, SKOPJE, NORTH MACEDONIA

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Abstract

Introduction: The management of acute lymphoblastic leukemia (ALL) has advanced substantially over the past decade, particularly with the integration of molecular and cytogenetic profiling. Identification of molecular markers at diagnosis is crucial for risk stratification, prognostication, and individualized treatment planning. Targeted therapies directed at specific molecular abnormalities have improved outcomes, especially in relapsed or refractory disease.

Aim: To analyze the molecular prognostic markers in patients diagnosed with ALL over four consecutive years at the University Clinic for Hematology, Skopje, and to evaluate their association with treatment outcomes and survival across different risk-stratified groups.

Material and methods: This retrospective study included newly diagnosed ALL patients treated at our centre between 2018 and 2022. Clinical, demographic, molecular, and treatment data were collected and analyzed. Outcomes included in-hospital mortality, remission rates, eligibility for allogeneic stem cell transplantation, transplantation outcomes, and overall survival. Survival analyses were performed according to initial molecular risk stratification.

Results: A total of 61 patients were diagnosed with ALL during the study period, with a median age at diagnosis of 42.9 years. B-cell ALL was identified in 38 patients (62.3%), T-cell ALL in 18 patients (29.5%), and biphenotypic leukemia in 5 patients (8.2%). Extramedullary disease was present at diagnosis in 9 patients (14.8%). High-risk disease was identified in 23 patients (37.7%). The most frequently used first-line therapy was the Hyper-CVAD protocol, administered to 41 patients (67%). Molecular analysis revealed BCR-ABL positivity in 10 patients (16.4%). Other detected abnormalities included RUNX1 mutation, NOTCH1 mutation and complex cytogenetic abnormalities. Allogeneic stem cell transplantation was performed in 12 patients (19.7%). Median overall survival was 17.5 months (data available for 58 patients). At last follow-up, 15 patients (25.9%) were alive.

Conclusion: Patients classified within the favorable ELN risk group demonstrated significantly longer survival compared to those with unfavorable molecular profiles. The observed rates of primary resistance and early mortality were consistent with published ELN data. These findings underscore the critical role of molecular diagnostics in guiding prognosis and therapeutic decision-making in ALL.

Keywords: acute lymphoblastic leukemia, molecular markers, risk stratification, stem cell transplantation

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous hematologic malignancy characterized by the clonal proliferation of immature lymphoid precursors in the bone marrow, peripheral blood, and extramedullary sites. Although ALL is most frequently diagnosed in children, adult cases account for a substantial proportion and are associated with less favorable outcomes. The biological diversity of ALL, reflected in its immunophenotypic, cytogenetic, and molecular features, has significant implications for prognosis and therapeutic decision-making.

Based on immunophenotypic characteristics, ALL is broadly classified into B-cell precursor ALL (BCP-ALL) and T-cell ALL (T-ALL), with a smaller subset of cases exhibiting mixed or biphenotypic features.

BCP-ALL represents the most common subtype in adults and encompasses a wide spectrum of genetic abnormalities, including recurrent chromosomal translocations and gene mutations that define distinct prognostic subgroups. Various developments in the past decade have led to improved outcomes in adults with BCP-ALL. These include the use of pediatric-like intensive chemotherapy regimens in adolescents and young adults,[1] the assessment of measurable residual disease (MRD) for prognostication and management decisions, and the development of immunotherapies[2]. Advances in the treatment of BCP-ALL have not translated into equivalent survival benefits for adult patients. Compared with children, adults more frequently harbor high-risk genetic abnormalities and are more susceptible to the toxic effects of intensive chemotherapy. While MRD-negative remission after induction therapy is an important favorable prognostic factor, it does not eliminate the risk of subsequent relapse.
T-ALL, although less frequent, is more commonly observed in younger male patients and is often associated with high tumor burden and mediastinal involvement.

Advances in molecular diagnostics have led to improved risk stratification and the incorporation of targeted therapies into treatment algorithms. Identification of specific molecular markers at diagnosis is now essential for predicting disease course, guiding treatment intensity, and selecting patients for allogeneic stem cell transplantation. In this context, evaluating the molecular landscape of ALL in real-world clinical settings remains crucial for optimizing patient outcomes. This has been shown in the review outlines of 25 years of advances in the understanding and management of Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph-positive ALL),[3] highlighting how the incorporation of tyrosine kinase inhibitors (TKIs), particularly in combination with bispecific antibodies like blinatumomab, has transformed what was once one of the most lethal hematologic malignancies into a disease with substantially improved long-term outcomes. This review article emphasizes the evolution of frontline and chemotherapy-free regimens that achieve high rates of molecular remission and durable survival, underscoring the impact of targeted therapy on both disease control and toxicity profiles.

Methods and materials

This retrospective, single-center cohort study included consecutive adult patients with newly diagnosed ALL treated at the University Clinic for Hematology, Skopje, North Macedonia, between January 2018 and December 2022. Diagnosis of ALL was established according to the World Health Organization (WHO) classification criteria, incorporating morphologic, immunophenotypic, cytogenetic, and molecular findings. Patients who lacked sufficient clinical or follow-up data were excluded from survival analyses.

Demographic, clinical, laboratory, treatment, and outcome data were extracted from institutional medical records. Collected variables included age at diagnosis, sex, immunophenotypic subtype (B-cell precursor ALL, T-cell ALL, or biphenotypic leukemia), presence of extramedullary disease at diagnosis, and comorbidities, which were categorized using the International Classification of Diseases, 10th revision (ICD-10) codes.

Molecular characterization was performed at diagnosis using standard-of-care techniques, including polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH), to detect recurrent genetic abnormalities relevant to ALL prognosis and risk stratification. Identified molecular markers were classified according to established prognostic models and European Leukemia Network (ELN) risk categories.

Patients were treated according to institutional protocols based on contemporary international guidelines, incorporating multi-agent chemotherapy regimens and targeted therapies when indicated. Treatment intensity and consolidation strategies, including eligibility for allogeneic hematopoietic stem cell transplantation (allo-HSCT), were determined by disease risk stratification, treatment response, and patient-related factors.

Primary outcome measures included in-hospital mortality, comorbidity burden, complete remission rates following induction therapy, rates of eligibility for allo-HSCT, transplantation-related outcomes, and overall survival. Overall survival was defined as the time from diagnosis to death from any cause or last follow-up.

Results

The primary focus of the analysis was the molecular profile of acute lymphoblastic leukemia (ALL) at the time of initial diagnosis. A total of 61 patients were diagnosed with ALL at the University Clinic for Hematology between 2018 and 2022. The median age at diagnosis was 42.9 years. Extramedullary disease at presentation was observed in 9 patients (14.8%). The cohort comprised 39 men (63.9%) and 22 women (36.1%) (Figure 1).

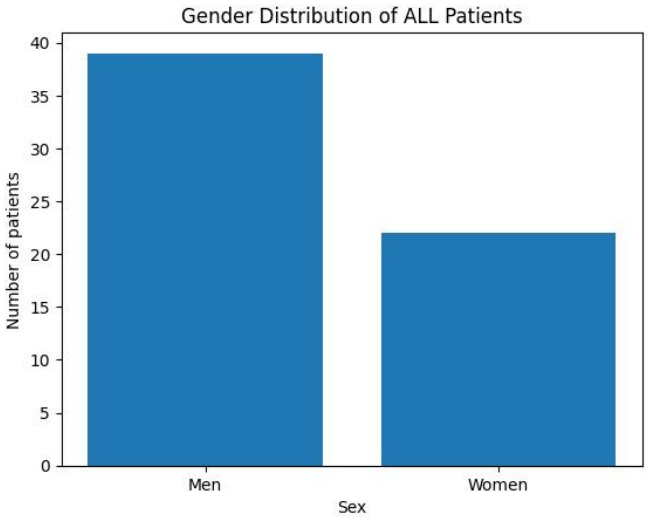

Fig. 1. Sex distribution of patients diagnosed with acute lymphoblastic leukemia

Geographic analysis revealed a marked clustering of patients in urban regions, with the highest concentration originating from Skopje, accounting for more than one third of cases. Additional clusters were observed in major regional centers including Tetovo, Kumanovo, Bitola, and Strumica. This distribution likely reflects population density and centralized referral to the national tertiary hematology center (Table 1).

Table 1. Geographic distribution of patients with ALL by city of residence

City	Number of patients
Skopje	23
Tetovo	6
Kumanovo	5
Bitola	4
Foreign country of residence	3
Gostivar	3
Strumica	3
Ohrid	3
Kavadarci	2
Veles	2
Shtip	1
Probishtip	1
Krushevo	1
Resen	1
Debar	1
Kochani	1
Valandovo	1
Struga	1

B-cell precursor ALL (B-ALL) was the most frequent subtype, diagnosed in 38 patients (62.3%). Among these, 22 patients were male (57.9%) and 16 were female (42.1%). T-cell ALL (T-ALL) was identified in 18 patients (29.5%), with a marked male predominance (14 men, 77.8%; 4 women, 22.2%), consistent with epidemiological data reported in the literature. All patients with T-ALL experienced death during the follow-up period, corresponding to a mortality rate of 100% in these patients. Although the number of T-ALL cases was limited, these findings suggest a markedly unfavorable outcome for T-ALL patients in this cohort and warrant cautious interpretation in the context of the small sample size. Biphenotypic leukemia was diagnosed in 5 patients (8.2%), of whom 3 were men (60%) and 2 were women (40%) (Figure 2).

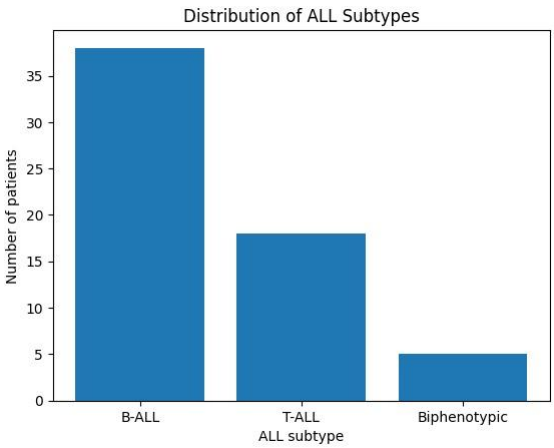

Fig. 2. Distribution of acute lymphoblastic leukemia subtypes in the study cohor

Based on molecular and clinical criteria, 23 patients (37.7%) were classified as having high-risk disease at diagnosis. The most administered first-line treatment was the Hyper-CVAD chemotherapy protocol, which was used in 41 patients (67%). BFM-based protocols, including both standard- and high-risk adaptations, collectively accounted for approximately 24% of the treatment approaches used.

Molecular analysis revealed BCR-ABL positivity in 10 patients (16.4%). Additional molecular abnormalities included RUNX1 mutation in one patient, del(1p32) in one patient, and a NOTCH1 mutation with concurrent complex cytogenetic abnormalities (19p deletion, 17p deletion, 14q deletion) and SF3B1 mutation in one patient (Table 3). Patients with adverse molecular features exhibited extremely poor survival, with deaths driven by both refractory disease and treatment-related complications.

Table 2. Baseline clinical and molecular characteristics of patients with ALL (2018-2022)

Characteristics	Value
Total patients	61
Median age at diagnosis, years	42.9
Male sex	39(63.9%)
Female sex	22(36.1%)
Extramedullary disease at diagnosis	9(14.8%)
B-cell precursor ALL	38(62.3%)
T-cell ALL	18(29.5%)
Biphenotypic ALL	5(8.2%)
High-risk disease	23(37.7%)
BCR-ABL positive	10(16.4%)
Allogeneic HSCT performed	12(19.7%)
Alive at last follow-up	15/58 (25.9%)

Table 3. Molecular abnormalities detected at diagnosis.

Molecular abnormality	n (%)
BCR-ABL	10(16.4%)
RUNX1 mutation	1(1.6%)
deletion (1p32)	1(1.6%)
NOTCH1 mutation with complex cytogenetics	1(1.6%)

Allogeneic hematopoietic stem cell transplantation was performed in 12 patients (19.7%). Median overall survival was 17.5 months, calculated for 58 patients with available follow-up data. At the time of analysis, 15 patients (25.9%) were alive (Table 4).

Table 4. Treatment and outcomes

Variable	n (%), months
Hyper-CVAD as first-line therapy	41(67%)
Allogeneic HSCT	12(19.7%)
Median overall survival (months)	17.5
Alive at last follow-up	15/58(25.9%)

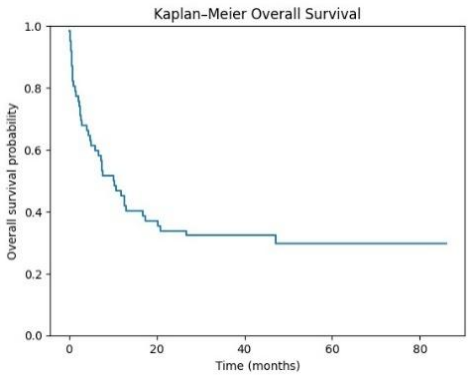

Fig. 3 Kaplan–Meier overall survival curve

Overall survival was defined as the interval from the date of diagnosis to death from any cause or last documented follow-up. Patients without a recorded date of death were censored at the time of last follow-up (April 2025).

The Kaplan-Meier survival curve demonstrates a pronounced early mortality, characteristic of adult acute lymphoblastic leukemia, occurring predominantly within the first year after diagnosis. This initial decline is followed by a stabilization of survival probabilities after approximately 25 months, with a distinct subset of patients achieving durable long-term survival extending beyond 5 to 7 years.

Overall survival was estimated using the Kaplan–Meier method, yielding a median overall survival of 17.5 months. These findings underscore the heterogeneous clinical course of adult ALL, marked by a substantial early risk and the potential for prolonged survival in patients who successfully overcome the initial high-risk period.

Discussion

This retrospective single-center study provides insight into the molecular characteristics, treatment approaches, and survival outcomes of adult patients diagnosed with acute lymphoblastic leukemia (ALL) in a real-world clinical setting. The findings highlight the marked biological heterogeneity of adult ALL, the high prevalence of adverse molecular features, and the persistence of early mortality despite the use of intensive chemotherapy and selective application of allogeneic hematopoietic stem cell transplantation. The observed association between molecular risk stratification and survival further confirms the central role of molecular diagnostics in guiding prognosis and therapeutic decisions in adult ALL.

In our cohort, B-cell precursor acute lymphoblastic leukemia was the most frequent immunophenotypic subtype, accounting for more than 60% of cases, while T-cell ALL and biphenotypic leukemia were less common[4-7]. This distribution is consistent with population-based studies and large adult ALL series, which report B-cell lineage disease as the predominant subtype in adults[4-7]. A clear male predominance was observed, particularly among patients with T-cell ALL, which is a well-established epidemiological feature and may reflect underlying biological differences in T-lineage leukemogenesis[6].

The median age at diagnosis of 42.9 years represents a relatively young adult population; however, outcomes in adults remain inferior compared with pediatric and adolescent patients [5,6]. This survival disadvantage has been attributed to age-related differences in disease biology, a higher frequency of adverse cytogenetic and molecular abnormalities, and reduced tolerance to intensive chemotherapy in adults[5,7]. Extramedullary involvement at diagnosis was present in approximately 15% of patients and was previously associated with increased disease burden and poorer prognosis in adult ALL[6,7].

Molecular profiling at diagnosis revealed BCR-ABL positivity in 16.4% of patients, a frequency within the range reported in European adult ALL cohorts[3]. Philadelphia chromosome positive ALL has historically been associated with poor outcomes; however, the introduction of tyrosine kinase inhibitors has resulted in significant improvements in remission rates and long-term survival[3]. Despite these advances, outcomes in routine clinical practice remain strongly influenced by early molecular diagnosis, access to targeted therapies, and the ability to intensify treatment when indicated.

Additional molecular abnormalities identified in our cohort included RUNX1 mutations, NOTCH1 mutations, and complex cytogenetic alterations. Although individually infrequent, these abnormalities carry important prognostic implications. RUNX1 mutations and complex karyotypes have been associated with genomic instability, resistance to chemotherapy, and inferior survival in adult ALL[8]. In contrast, the prognostic impact of NOTCH1 mutations, particularly in T-cell ALL, appears to be variable and dependent on

coexisting genetic abnormalities, underscoring the importance of integrated molecular risk assessment rather than reliance on single molecular markers[9].

The Hyper-CVAD regimen was the most used first-line therapy in our cohort, reflecting its long-standing role as an intensive chemotherapy backbone in adult ALL[10,11]. Although Hyper-CVAD is associated with high initial remission rates, its long-term effectiveness is limited in patients with adverse molecular features, and treatment-related toxicity remains substantial[11,12]. These limitations are particularly relevant in older patients and in those with significant comorbidities, who may be unable to tolerate repeated cycles of intensive chemotherapy.

The outcomes observed in this study are consistent with historical adult ALL series, in which conventional chemotherapy alone failed to provide durable survival benefits in high-risk patients[10,11]. These findings further support the need for risk-adapted treatment strategies that incorporate molecularly targeted agents and immunotherapy earlier in the disease course.

Comorbid conditions present at the time of diagnosis were common in this cohort and predominantly involved chronic cardiovascular, metabolic, and systemic disorders. Based on the ICD-10 classification, the most frequently reported comorbidities corresponded to diseases of the circulatory system (I00-I99; including arterial hypertension and other cardiovascular diseases), endocrine and metabolic disorders (E00-E90; predominantly diabetes mellitus), and chronic diseases of other organ systems, such as respiratory (J00-J99) and renal disorders (N00-N99).

The presence of comorbidities at diagnosis was strongly associated with mortality in this evaluated cohort. Among patients with at least one documented comorbidity, nearly all experienced death during follow-up, indicating an exceptionally high mortality rate in this subgroup. Although causality cannot be inferred, this observation suggests that baseline comorbid conditions may have significantly contributed to reduced treatment tolerance, increased susceptibility to treatment-related complications-particularly infections-and overall poor outcomes. These findings highlight the clinical relevance of comorbidity burden at diagnosis and underscore the importance of comprehensive baseline assessment and risk stratification in patients with acute lymphoblastic leukemia, as reported in other relevant studies available.

Allogeneic hematopoietic stem cell transplantation was performed in approximately 20% of patients, despite more than one third being classified as high risk. This discrepancy reflects the practical limitations of transplantation in real-world settings, including early disease progression, treatment-related mortality, patient comorbidities, and donor availability. While allo-HSCT remains the most effective consolidative strategy for high-risk adult ALL, its benefit depends on achieving remission and surviving the initial phase of intensive therapy[12,13].

Previous studies have demonstrated that transplantation provides the greatest benefit in patients with adverse cytogenetic features or persistent measurable residual disease following induction therapy[12-14]. In our cohort, early mortality likely prevented a proportion of potentially eligible patients from proceeding to transplantation, highlighting the need for safer and more effective induction strategies.

The median overall survival of 17.5 months observed in this study is comparable to outcomes reported in population-based analyses of adult ALL outside of clinical trial settings[15]. Kaplan-Meier analysis demonstrated a pronounced decline in survival during the first year after diagnosis, reflecting the combined effects of aggressive disease biology and treatment-related toxicity. This pattern of early mortality has been consistently reported in adult ALL and remains a major barrier to improving long-term survival[5,15].

After approximately two years, the survival curve showed relative stabilization, with a subset of patients achieving long-term remission extending beyond five years. This plateau

suggests that durable disease control is achievable in selected patients, particularly those with favorable molecular profiles and adequate tolerance to intensive therapy.

The leading causes of death in this cohort were infectious complications, most commonly sepsis, followed by disease progression or refractory leukemia. Transplant-related complications and multi-organ failure represented additional major contributors to mortality. Bleeding events were observed less frequently. Overall, the pattern of mortality reflects a combination of aggressive disease biology and treatment-related toxicity in a predominantly high-risk population. These findings highlight the critical impact of infection risk and transplant-associated morbidity on overall survival and underscore the importance of optimized supportive care and risk-adapted therapeutic strategies, in line with ELN recommendations.

Recent advances in ALL treatment have significantly altered the therapeutic landscape, particularly through the introduction of immunotherapy and treatment strategies guided by measurable residual disease assessment. Blinatumomab has been shown to improve overall survival and reduce relapse rates compared to conventional chemotherapy in both MRD-negative and advanced disease settings[1]. In addition, chemotherapy-free or low-intensity regimens combining tyrosine kinase inhibitors with immunotherapy demonstrated promising results in Philadelphia chromosome–positive ALL[3].

The outcomes observed in the present study highlight the gap between results achieved in clinical trials and those observed in routine clinical practice. Limited access to novel agents, delays in molecular diagnostics, and healthcare system constraints may all contribute to less favorable real-world outcomes. Broader availability of targeted therapies and routine incorporation of MRD assessment into standard treatment algorithms are likely to be essential steps toward improving outcomes in adult ALL.

Conclusion

This study highlights the pronounced molecular heterogeneity and unfavorable prognosis of adult acute lymphoblastic leukemia in a real-world clinical setting. Molecular risk stratification at diagnosis was strongly associated with survival outcomes, underscoring its critical role in guiding therapeutic decision-making. Despite the use of intensive chemotherapy and selective application of allogeneic stem cell transplantation, early mortality remained substantial, limiting long-term survival. These findings emphasize the need for earlier integration of targeted and immunotherapeutic approaches and broader implementation of risk-adapted treatment strategies to improve outcomes in adult ALL.

Conflict of interest statement. None declared.

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