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Genetic Insights and Clinical Implications in the Diagnosis of Acute Myeloid Leukemia: An Updated Perspective

Elahe Razmara Lak¹ , Aziz Eghbali¹ , Omid Kiani Ghalesardi² , Nafiseh Mortazavi^{3*}

1. Clinical Research Development Center of Aliasghar Hospital, Iran University of Medical Sciences, Tehran, Iran.

2. Department of Hematology and Blood Banking, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.

3. Department of Pathology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Pediatric acute myeloid leukemia (AML) is biologically heterogeneous, necessitating integrated genetic and immunophenotypic profiling for precise diagnosis and risk stratification. We analyzed 74 pediatric AML patients diagnosed between 2012 and 2023 at Ali-Asghar Children's Hospital, Tehran, Iran, via blood counts, bone marrow morphology, cytogenetic karyotyping, flow cytometry, and nested PCR for common fusion genes. In this study, the median age was 5.9 years (range, 0.5–17 years). Clinical presentations vary by cytogenetic subtype: t(15;17) is associated with bleeding, bruising, and fever; t(8;21) is associated with moderate fever and fatigue; inv(16) is associated with fatigue and minimal bleeding; trisomy 19 and duplication 5q often lack systemic symptoms; and cytogenetically normal cases present diverse symptoms, including fever, fatigue, weakness, and weight loss. The most frequent rearrangements were t(8;21) (n=9, 12.16%), t(15;17) (n=8, 10.81%), and t(9;11) (n=8, 10.81%), whereas t(1;22) (n=2, 2.70%) and inv(16) (n=1, 1.35%) were rare. Immunophenotyping revealed universal CD33 and CD45 expression (>90%), frequent CD34 positivity, the absence of HLA-DR and CD11b at t(15;17), and characteristic CD34/CD33 patterns at t(8;21). Our findings underscore the genetic and immunophenotypic complexity of pediatric AML and highlight the value of integrated diagnostics for risk-adapted therapy. Personalized treatment strategies may improve outcomes. However, multicenter studies are needed to validate these findings and identify novel therapeutic targets.

*Corresponding:

Nafiseh Mortazavi

Address:

Department of Pathology,
School of Medicine, Iran
University of Medical Sciences,
Tehran, Iran.

E-mail:

nafiseh.mortazavi@yahoo.com

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by uncontrolled proliferation of abnormal myeloid cells in the bone marrow and peripheral blood (1). The 2022 World Health Organization (WHO) classification of hematolymphoid tumors represents a pivotal advancement in the diagnostic framework for AML, underscoring the central role of genetic and molecular abnormalities in disease definition (1-3). This paradigm shift reflects a broader movement within hematologic oncology toward genomically driven classifications, superseding the traditional reliance on morphologic and cytochemical assessments (1, 2).

The WHO 2022 classification aligns closely with the International Consensus Classification (ICC), which similarly prioritizes molecular aberrations in delineating AML subtypes, although minor differences in nomenclature persist between the two systems (2, 3). Both frameworks aim to increase diagnostic precision and inform individualized treatment strategies by incorporating specific genetic alterations—such as chromosomal translocations, inversions (Inv), and mutations affecting key hematopoietic regulatory pathways—into the diagnostic criteria. This integration of genetic insights into clinical practice not only refines disease classification but also facilitates the development of personalized therapeutic approaches, thereby improving patient outcomes (1, 4).

The incidence of AML increases with age, peaking in adults over 60 years. However, AML remains a significant cause of childhood leukemia, accounting for approximately 15–20% of all leukemia cases in pediatric populations (5, 6). Cytogenetic abnormalities are crucial in AML diagnosis, as they influence both prognosis and treatment decisions. The most common cytogenetic abnormalities in AML include t(8;21), inv(16), and t(15;17), each of which is associated with distinct clinical features and outcomes. In addition to these chromosomal changes, molecular mutations such as Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) also play critical roles in disease behavior and prognosis. For example, t(8;21) is typically associated with a favorable response to chemotherapy, whereas FLT3-ITD mutations are linked to more aggressive disease and poorer outcomes (6-8). The 2022 WHO classification provides a detailed framework for identifying these abnormalities and

stratifying patients into risk groups on the basis of their genetic profile (4).

Recent multiomics studies, such as that of Patel et al. (2012) (9), have substantially expanded our understanding of AML by integrating genomic, transcriptomic, and epigenetic landscapes to define novel molecular subtypes and identify potential therapeutic targets. These large-scale, multi-institutional efforts provide a system-level perspective on AML pathogenesis. However, they may lack the clinical granularity and contextual depth afforded by focused, single-center investigations. In contrast, our study employs a cytogenetic-centric approach within a single clinical institution, enabling a detailed evaluation of the associations between specific chromosomal abnormalities and clinical outcomes in pediatric AML patients within a controlled and uniform clinical setting.

While next-generation sequencing (NGS) technologies have revolutionized AML research, conventional cytogenetics remains a fundamental component of diagnostic and prognostic algorithms, particularly in pediatric populations and resource-constrained environments where comprehensive multiomics profiling may not be routinely feasible. By delivering clinically actionable insights on the basis of established cytogenetic markers, our study complements broader multiomics research, offering a pragmatic and immediately translatable framework for risk stratification and individualized treatment planning in pediatric AML—a population in which disease biology and therapeutic responses often diverge from those in adult cases.

The prognosis of patients with AML is strongly influenced by genetic factors, with certain cytogenetic abnormalities correlated with distinct outcomes; notably, patients with t(15;17), characteristic of acute promyelocytic leukemia (APL), often achieve favorable responses to all-trans retinoic acid (ATRA) and arsenic trioxide therapy, which has demonstrated noninferiority and potential superiority over standard ATRA plus chemotherapy, yielding a 97% event-free survival rate versus 85%, with reduced hematologic toxicity and comparable antileukemic efficacy, thereby supporting the effectiveness of a chemotherapy-free regimen in patients with APL (10).

In contrast, FLT3 mutations and complex cytogenetic abnormalities are associated with poor prognosis and require more intensive chemotherapy

and novel targeted therapies (11). Risk stratification on the basis of cytogenetics allows for a more tailored approach to therapy, improving survival rates in specific patient subsets. However, despite advances in treatment, a significant proportion of patients relapse or fail to respond to therapy, highlighting the need for novel treatments and more refined diagnostic tools (12). AML is often characterized by a range of complications, primarily due to its impact on hematopoiesis. Symptoms typically include fatigue, fever, bruising, and bleeding, which result from anemia, thrombocytopenia, and neutropenia (13).

Specific complications also vary depending on the type of AML. For example, APL associated with t(15;17) is frequently complicated by disseminated intravascular coagulation (DIC), a life-threatening condition requiring urgent intervention (14). In contrast, AML with inv(16)(p13.1q22), marked by the CBFB-MYH11 fusion, is a distinct subtype associated with favorable outcomes and high remission rates, particularly with high-dose cytarabine (15).

It often presents as acute myelomonocytic leukemia with abnormal eosinophils. The prognosis may worsen with additional abnormalities, such as TP53 mutations, complex karyotypes, or trisomy 8/22, especially in pediatric M4EO patients. KIT mutations and high leukocyte counts at diagnosis also negatively impact outcomes (16, 17). Single-cell RNA studies have revealed cellular heterogeneity and dysregulated inflammatory and metabolic pathways (18). Identifying these complications early is critical for improving patient outcomes.

Our study aimed to elucidate the clinical and genetic landscape of pediatric AML. By investigating the correlation between cytogenetic abnormalities and clinical outcomes, we hope to contribute to a deeper understanding of AML genetic diversity, its impact on prognosis, and the potential for personalized treatment strategies. Given the heterogeneity of AML, our findings have the potential to influence risk stratification and the selection of therapies tailored to individual genetic profiles, ultimately improving patient survival rates and quality of life.

Methods

Patients and Study Design

This retrospective cross-sectional study enrolled 74 pediatric AML patients (aged 1–18 years) referred

to Ali-Asghar Children Hospital (Tehran, Iran) between 2012 and 2023. The diagnosis of AML was established in accordance with the 2022 WHO classification, which integrates clinical presentation, hematologic parameters, cytogenetic findings, and immunophenotypic profiles.

Ethical approval (IR.IUMS.REC.1403.632) was obtained from Iran University of Medical Sciences. Written informed consent was obtained from the legal guardians of all participants, with additional assent from patients aged ≥ 12 years.

Cell Counting and Flow Cytometry

Peripheral blood samples (3 mL) were collected into EDTA tubes containing 1.5 mg/mL anticoagulant. Complete blood counts (CBCs) were performed via a Sysmex KX-21 automated hematology analyzer, which was calibrated daily via manufacturer-supplied control materials to ensure accuracy and consistency. Immunophenotypic analysis was conducted via flow cytometry using a BD FACSCalibur™ system. A comprehensive panel of 27 cluster of differentiation (CD) markers (Exbio), including CD7, CD19, and CD79a, was employed to facilitate thorough AML characterization.

Cytogenetic analysis

Bone marrow cultures (37°C, 5% CO₂) were synchronized with methotrexate (10 μ M, 3–5 hours) and released with thymidine (10 μ M, 17 hours). Colchicine (0.1 μ g/mL, 30 minutes) arrested metaphases. Chromosomal banding was performed via Wright–Giemsa staining, and karyotypes were analyzed with IKAROS software (v5.3, Metasystem). Clonal abnormalities required ≥ 2 structural or ≥ 3 numerical concordant metaphases.

RNA Preparation and Nested Polymerase Chain Reaction (PCR)

Total RNA was extracted via TRIzol® (Invitrogen), treated with DNase I (Qiagen), and quantified via NanoDrop™. cDNA synthesis was performed with random hexamers (Thermo Fisher) and M-MLV reverse transcriptase.

Nested PCR (Bio-Rad T100™) targeted WHO-defined translocations (e.g., RUNX1-RUNX1T1). The controls included HL-60 (positive) and no-template (negative) samples.

Immunohistochemistry

MPO and lysozyme staining were performed with Dako antibodies (1:100, 1 hour, RT). Antigen retrieval (citrate buffer, pH 6.0) preceded HRP-DAB visualization. PAS staining was performed according to standard protocols.

Statistical analysis

Data normality was evaluated via the Shapiro–Wilk test. Descriptive statistics are reported as the means \pm standard deviations (SDs) or medians, as appropriate. Inferential analyses were performed via either the independent samples t test or the Mann–Whitney U test, depending on the data distribution. All the statistical computations were conducted via SPSS software, version 20 (IBM Corp.), with a significance threshold set at $\alpha = 0.05$.

Results

Patient Demographics and Clinical Characteristics

The patient cohort in this study comprises 74 individuals diagnosed with AML, categorized by distinct cytogenetic abnormalities, which are known to play a crucial role in prognostication and treatment decisions. The detailed demographic and laboratory characteristics of the patients included in the study are summarized in Table 1.

Age distribution

The mean age of the cohort was 5.9 ± 5.0 years, with a broad range of 0.08 to 18.0 years, indicating that the cohort spans a pediatric and adolescent population

(Figure1). The mean age for patients with t(8;21) abnormalities was slightly older at 8.3 ± 5.3 years, whereas patients with t(9;11) and t(1;22) abnormalities were younger, with mean ages of 3.2 ± 4.5 years and 3.5 ± 3.5 years, respectively. The wide range of ages reflects the heterogeneous nature of AML in children and adolescents, with early diagnosis and treatment playing a crucial role in disease management.

Body mass index (BMI)

The BMI data for this study revealed a mean BMI of 15.2 ± 3.2 , with a range from 6.9 to 29.1. These values indicate that many patients present with a lower BMI, which is often observed in children undergoing treatment for serious hematologic diseases such as AML. Notably, patients with t(1;22) had the highest mean BMI (21.0 ± 7.1), whereas patients with t(9;11) had the lowest mean BMI (13.7 ± 2.2), potentially reflecting the nutritional impact of the disease and its treatments.

White blood cell (WBC) counts

The mean WBC count across all patients was $44.8 \pm 55.2 \times 10^9/L$, with a wide range from 1.3 to $205.2 \times 10^9/L$. The elevated WBC counts observed in many patients indicate active leukocytosis, a hallmark of AML. The t(9;11) group presented the highest mean WBC ($73.5 \pm 78.1 \times 10^9/L$), which may reflect a more aggressive disease presentation, whereas the t(15;17) group presented a more moderate mean WBC of $25.8 \pm 31.8 \times 10^9/L$. The variability in WBC count among the groups underscores the heterogeneity of AML and the varying severity of the disease at diagnosis (Figure 2).

Table 1. Summary of Patient Demographics and Laboratory Characteristics

AML with Cytogenetic Abnormality	Number of Patients (%)	Age \pm SD (range)	BMI \pm SD (range)	WBC \pm SD (range)	HB \pm SD (range)	PLT \pm SD (range)
No Detected Cytogenetic Abnormality	24 (32.43)	5.9 ± 5.0 (0.08 - 18.0)	15.2 ± 3.2 (6.9 - 29.1)	44.8 ± 55.2 (1.3 - 205.2)	8.1 ± 2.2 (3.1 - 13.3)	68.2 ± 74.4 (7 - 342)
t(8;21)	9 (12.16)	8.3 ± 5.3 (0.08 - 18.0)	16.3 ± 2.4 (6.9 - 29.1)	11.9 ± 9.9 (1.3 - 205.2)	7.9 ± 3.0 (3.1 - 13.3)	81.0 ± 81.5 (7 - 342)
t(15;17)	8 (10.81)	11.1 ± 3.9 (0.08 - 18.0)	16.1 ± 2.2 (6.9 - 29.1)	25.8 ± 31.8 (1.3 - 205.2)	7.6 ± 1.3 (3.1 - 13.3)	41.6 ± 35.4 (7 - 342)
t(9;11)	8 (10.81)	3.2 ± 4.5 (0.08 - 18.0)	13.7 ± 2.2 (6.9 - 29.1)	73.5 ± 78.1 (1.3 - 205.2)	7.7 ± 1.0 (3.1 - 13.3)	70.6 ± 71.5 (7 - 342)

AML with Cytogenetic Abnormality	Number of Patients (%)	Age \pm SD (range)	BMI \pm SD (range)	WBC \pm SD (range)	HB \pm SD (range)	PLT \pm SD (range)
t(1;22)	2 (2.70)	3.5 \pm 3.5 (0.08 - 18.0)	21.0 \pm 7.1 (6.9 - 29.1)	46.6 \pm 63.1 (1.3 - 205.2)	7.1 \pm 1.3 (3.1 - 13.3)	230.0 \pm 158.4 (7 - 342)
t(17;19)	1 (1.35)	5.0 (0.08 - 18.0)	12.0 (6.9 - 29.1)	60.5 (1.3 - 205.2)	11.7 (3.1 - 13.3)	333.0 (7 - 342)
t(9;12)	1 (1.35)	1.0 (0.08 - 18.0)	15.6 (6.9 - 29.1)	18.7 (1.3 - 205.2)	9.4 (3.1 - 13.3)	204.0 (7 - 342)
t(8;21), del 7	1 (1.35)	11.0 (0.08 - 18.0)	24.9 (6.9 - 29.1)	7.1(1.3 - 205.2)	6.8 (3.1 - 13.3)	110.0 (7 - 342)
t(8;16)	1 (1.35)	12.0 (0.08 - 18.0)	29.1 (6.9 - 29.1)	3.3(1.3 - 205.2)	5.2 (3.1 - 13.3)	25.0 (7 - 342)
t(6;9)	1 (1.35)	15.0 (0.08 - 18.0)	20.3 (6.9 - 29.1)	18.9 (1.3 - 205.2)	7.8 (3.1 - 13.3)	50.0 (7 - 342)
t(6;11)	1 (1.35)	9.0 (0.08 - 18.0)	14.8 (6.9 - 29.1)	19.2 (1.3 - 205.2)	7.9 (3.1 - 13.3)	37.0 (7 - 342)
t(4;6), inv6, Trisomi 21	1 (1.35)	2.0 (0.08 - 18.0)	8.2 (6.9 - 29.1)	83.0 (1.3 - 205.2)	6.4 (3.1 - 13.3)	10.0 (7 - 342)
t(4;11)	1 (1.35)	0.2 (0.08 - 18.0)	6.9 (6.9 - 29.1)	127.0 (1.3 - 205.2)	8.0 (3.1 - 13.3)	45.0 (7 - 342)
t(3;3)	1 (1.35)	1.0 (0.08 - 18.0)	13.2 (6.9 - 29.1)	16.1 (1.3 - 205.2)	10.1 (3.1 - 13.3)	100.0 (7 - 342)
16P	1 (1.35)	13.0 (0.08 - 18.0)	14.6 (6.9 - 29.1)	5.8 (1.3 - 205.2)	8.4 (3.1 - 13.3)	108.0 (7 - 342)
INV 16, Trisomy 21	1 (1.35)	15.0 (0.08 - 18.0)	14.8 (6.9 - 29.1)	4.2 (1.3 - 205.2)	7.2 (3.1 - 13.3)	12.0 (7 - 342)
t(12;21), add 6, add11	1 (1.35)	5.0 (0.08 - 18.0)	14.5 (6.9 - 29.1)	1.3 (1.3 - 205.2)	10.5 (3.1 - 13.3)	28.0 (7 - 342)
t(11;17), Trisomi 21, del16	1 (1.35)	1.0 (0.08 - 18.0)	13.0 (6.9 - 29.1)	12.6 (1.3 - 205.2)	7.6 (3.1 - 13.3)	31.0 (7 - 342)
inv16	1 (1.35)	12.0 (0.08 - 18.0)	16.7 (6.9 - 29.1)	14.9 (1.3 - 205.2)	8.4 (3.1 - 13.3)	147.0 (7 - 342)
del7, del10, add16	1 (1.35)	12.0 (0.08 - 18.0)	14.9 (6.9 - 29.1)	1.6 (1.3 - 205.2)	8.9 (3.1 - 13.3)	46.0 (7 - 342)
del12p, add7	1 (1.35)	2.0 (0.08 - 18.0)	13.0 (6.9 - 29.1)	14.6 (1.3 - 205.2)	5.2 (3.1 - 13.3)	36.0 (7 - 342)
del11, t(4;11)	1 (1.35)	13.0 (0.08 - 18.0)	15.2 (6.9 - 29.1)	27.0 (1.3 - 205.2)	8.3 (3.1 - 13.3)	20.0 (7 - 342)
del 7q. Inv 16	1 (1.35)	7.0 (0.08 - 18.0)	15.1 (6.9 - 29.1)	44.9 (1.3 - 205.2)	4.4 (3.1 - 13.3)	33.0 (7 - 342)

AML with Cytogenetic Abnormality	Number of Patients (%)	Age ± SD (range)	BMI ± SD (range)	WBC ± SD (range)	HB ± SD (range)	PLT ± SD (range)
del 7, Extra 12	1 (1.35)	10.0 (0.08 - 18.0)	11.7 (6.9 - 29.1)	41.3 (1.3 - 205.2)	6.0 (3.1 - 13.3)	10.0 (7 - 342)
add 2p, add3q, add7, add10, add19, del8	1 (1.35)	1.0 (0.08 - 18.0)	17.3 (6.9 - 29.1)	15.4 (1.3 - 205.2)	7.4 (3.1 - 13.3)	30.0 (7 - 342)
add 17. inv16	1 (1.35)	4.0 (0.08 - 18.0)	11.7 (6.9 - 29.1)	132.1 (1.3 - 205.2)	5.2 (3.1 - 13.3)	68.0 (7 - 342)
Trisomy 19, duplication 5q	1 (1.35)	7.0 (0.08 - 18.0)	12.5 (6.9 - 29.1)	11.0 (1.3 - 205.2)	7.9 (3.1 - 13.3)	64.0 (7 - 342)
t(X;16), Trisomy 21	1 (1.35)	14.0 (0.08 - 18.0)	24.9 (6.9 - 29.1)	14.5 (1.3 - 205.2)	8.2 (3.1 - 13.3)	42.0 (7 - 342)

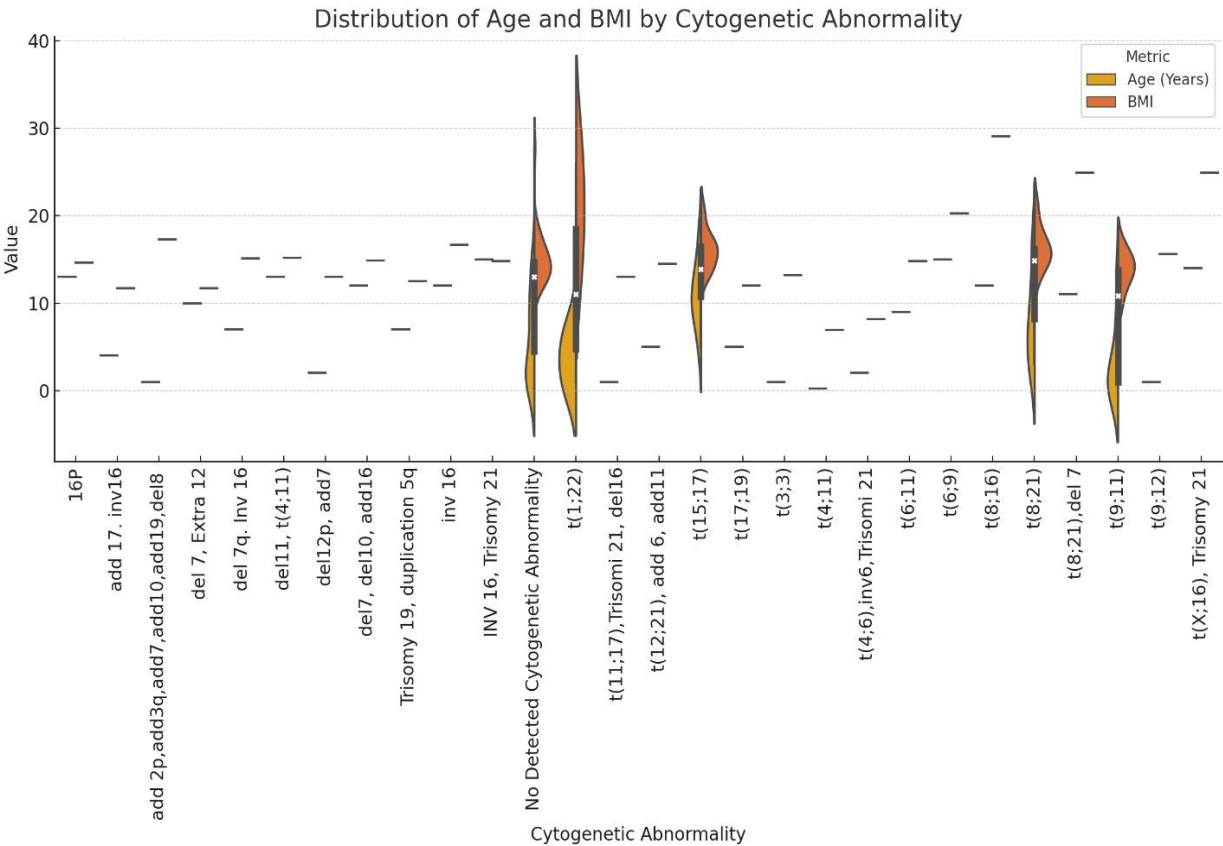


Figure 1. Violin plots illustrating the distributions of age and BMI among AML patients stratified by cytogenetic abnormalities. Each violin corresponds to a specific cytogenetic subgroup, with the left and right halves representing Age and BMI distributions, respectively. The width of each segment reflects the kernel density estimate, where broader regions indicate higher concentrations of data points. The median value for each parameter is denoted by a central horizontal line within each violin. The number of patients in each cytogenetic subgroup is indicated adjacent to the corresponding label.

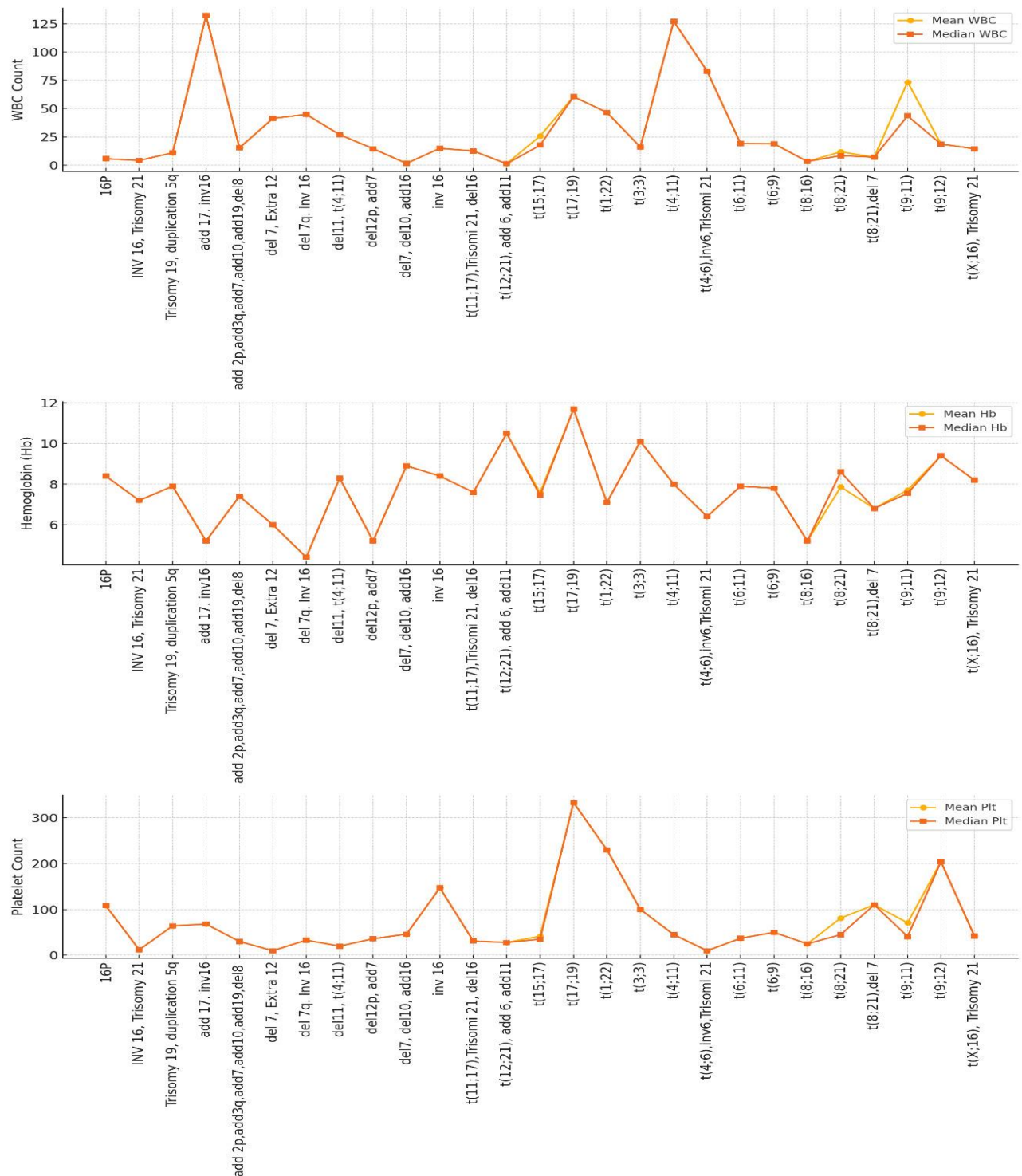


Figure 2. Trends in the WBC count, Hb level, and PLT among AML patients stratified by cytogenetic abnormalities. Each panel presents both the mean and median values for the respective hematologic parameter across distinct cytogenetic subgroups. The WBC panel highlights variations in leukocyte burden associated with specific genetic profiles. The Hb panel illustrates differences in anemia severity among the cytogenetic groups, whereas the PLT panel demonstrates variations in PLT counts, reflecting potential differences in thrombopoiesis and coagulopathy risk. The X-axis denotes cytogenetic abnormalities, and the Y-axis corresponds to the measured laboratory values for each parameter. The markers represent the mean and median values, facilitating comparisons across genetic subtypes.

Hemoglobin (Hb) levels

The mean Hb level for the cohort was 8.1 ± 2.2 g/dL, with a range from 3.1 to 13.3 g/dL, reflecting significant anemia, a common finding in AML patients due to bone marrow failure. The t(9;11) and t(1;22) groups presented similar Hb levels (7.7 ± 1.0 and 7.1 ± 1.3 , respectively), suggesting a marked reduction in red blood cell production, whereas the t(8;21) and t(15;17) groups presented slightly greater Hb levels (7.9 ± 3.0 and 7.6 ± 1.3 , respectively).

Platelet (PLT) count

PLT counts were found to be low across the cohort, with a mean PLT of $68.2 \pm 74.4 \times 10^9/L$ and a range from 7 to $342 \times 10^9/L$, indicating thrombocytopenia, a common complication in AML. The t(1;22) group had the highest mean PLT ($230.0 \pm 158.4 \times 10^9/L$), which might reflect differences in bone marrow infiltration or response to treatment. In contrast, the t(15;17) group had a much lower mean PLT ($41.6 \pm 35.4 \times 10^9/L$), which may be correlated with the disease's impact on PLT production (Figure 2).

Signs and symptoms

In this study, we analyzed a range of symptoms that are commonly associated with AML, including fever, fatigue, bruising, weakness, weight loss, and infection (Figure 3).

Patients classified under the "No Detected Cytogenetic Abnormality" group exhibited a broad range of symptoms, reflecting the diversity of disease presentations in this subgroup. Fever was one of the most prevalent symptoms, appearing in a significant proportion of these patients. Additionally, a notable percentage of patients reported fatigue, weakness, and weight loss. These symptoms suggest that the absence of a detectable cytogenetic abnormality does not necessarily correlate with a mild disease presentation; rather, it points to the possibility that other underlying molecular or environmental factors contribute to disease manifestation. Fever, along with symptoms such as weakness and weight loss, is often observed in patients with systemic involvement and is commonly observed in more aggressive forms of leukemia.

This group also had a more diverse presentation of symptoms, indicating that the lack of a specific cytogenetic abnormality may result in a wide variety of clinical manifestations. In contrast, patients with trisomy 19 and duplication 5q displayed a much lower

prevalence of symptoms, with many key signs such as fever, bruising, and significant weight loss absent. The findings from this group suggest that these patients may present with less aggressive forms of AML, where the disease does not immediately result in systemic manifestations. This could be indicative of a more indolent disease course or early-stage leukemia, where symptoms are either subtle or develop over a longer period. The relatively lower incidence of symptoms such as fatigue and weight loss in this group further supports this notion of a milder presentation.

On the other hand, patients with the inv (16) translocation presented a greater prevalence of symptoms, particularly fatigue, which was reported by all patients in this group. The inv (16) translocation is known to be associated with a specific subtype of AML that tends to involve a high tumor burden and bone marrow infiltration.

This could explain the frequent reporting of fatigue, as bone marrow failure or infiltration by leukemic cells can lead to a decrease in normal blood cell production, resulting in general malaise and tiredness. Interestingly, other symptoms, such as bruising and bleeding, were largely absent in this group, which may suggest that while the disease is aggressive in nature, it does not immediately result in severe thrombocytopenia or associated bleeding tendencies. This is in stark contrast to other cytogenetic groups, where severe cytopenia often leads to more noticeable bleeding and bruising.

Similarly, patients with the t(8;21) translocation (RUNX1-RUNX1T1) presented a moderate prevalence of fever and fatigue. Fever was reported in 11.1% of patients, and fatigue was also prevalent at a similar rate. These symptoms are consistent with a diagnosis of AML with t(8;21), a chromosomal abnormality that is often linked to a favorable prognosis in AML patients but still leads to some degree of systemic symptoms. While these symptoms were present, they were not as frequent or severe as those seen in other cytogenetic subgroups, reinforcing the idea that patients with this translocation typically fare better in terms of symptom severity and overall disease course.

Interestingly, groups with more complex cytogenetic abnormalities, such as those involving additions (add) to chromosomes 2p and 7q and deletions (del) on chromosome 7, presented relatively few symptoms. This could reflect the fact that these cytogenetic abnormalities may be associated with less

aggressive forms of AML or a disease stage that does not immediately result in significant clinical manifestations. In these patients, fatigue and weight loss were rare, suggesting that these patients may present at an earlier stage or with a disease burden that is not yet sufficient to cause systemic signs.

The group with the t(15;17) translocation, which is typically associated with APL, displayed a higher prevalence of bleeding symptoms, including bruising and fever. APL is known for its association with DIC, which can cause severe bleeding and clotting issues, explaining the higher rate of bruising and other bleeding-related symptoms in these patients. The presence of fever in this group aligns with the known clinical presentation of APL, where fever is often a presenting symptom in the acute phases of the disease, particularly when complications such as infection or DIC occur. The analysis of the prevalence of symptoms among AML patients with different cytogenetic abnormalities revealed important differences in the clinical manifestations associated with each cytogenetic group. These findings underscore the complexity and heterogeneity of AML, where the presence or absence of specific genetic abnormalities can have a profound impact on the nature of symptoms and the overall clinical presentation.

Patients with "No Detected Cytogenetic Abnormality" presented the broadest range of symptoms, whereas more specific genetic alterations, such as trisomy 19 or inv(16), were associated with milder symptomatology. Conversely, translocations such as t(15;17) and t(8;21) were linked with specific symptoms such as fever and fatigue, and in the case of APL, they included bleeding and bruising. These insights are crucial for understanding the clinical spectrum of AML.

Cytogenetic Abnormalities

Cytogenetic analysis of the AML cohort revealed a diverse spectrum of chromosomal abnormalities. Notably, a substantial subset of patients (n = 24, 32.43%) presented no detectable cytogenetic aberrations. Among patients with identifiable abnormalities, several recurrent translocations have emerged as particularly prevalent. The translocation t(8;21) was the most frequent, identified in 12.16% of the cases (n = 9). This aberration is classically associated with the AML-M2 (myeloblastic) subtype. The t(15;17) translocation, detected in 10.81% of

patients (n = 8), was strongly correlated with acute promyelocytic leukemia (APL). Similarly, t(9;11) translocation occurred in 10.81% of the cases (n = 8) (Figure 4).

In addition to these common translocations, rarer cytogenetic alterations were detected. These included t(1;22) (n = 2, 2.70%) and inv(16) (n = 1, 1.35%), as well as complex abnormalities such as t(4;6), inv(6), and trisomy 21 (n = 1, 1.35% each). Furthermore, the co-occurrence of multiple cytogenetic abnormalities, such as t(8;21) with del(7) or t(9;12), was observed in individual AML patients. The data also included several patients with nonspecific cytogenetic abnormalities, such as del 7q, Inv 16 and add 2p, add3q, add7, add10, add19, del8, which may reflect clonal evolution or be indicative of more aggressive disease features. These findings underscore the heterogeneity in the AML genetic landscape and reinforce the importance of cytogenetic testing in diagnosing and determining the prognosis of AML patients.

Immunophenotypic marker expression

The analysis of immunophenotypic marker expression in AML patients revealed variable levels of expression across multiple markers (Fig 5). The markers analyzed included CD11a, CD11b, CD11c, CD18, CD34, CD36, CD41, CD42b, CD46, CD61, CD64, HLA-DR, CD117, CD58, CD123, CD66c, CD13, CD14, CD15, CD33, CD38, CD45, CD56, CD235a, iMPO, PAS, and lysozyme (Fig 5). Notably, CD34 had a mean expression of 38.25, with a maximum of 99.0, indicating that a significant portion of AML blasts retain progenitor cell-like characteristics. Similarly, HLA-DR had a mean expression of 48.44, which is consistent with the role of HLA-DR in immune responses and its implication in the immune evasion mechanisms of AML. CD33 had a mean expression of 57.74, further supporting the myeloid origin of the AML cells in the study population.

CD45 demonstrated a higher mean expression of 74.57, with some patients showing near-maximal expression (100.0). These findings suggest that a substantial proportion of leukemic cells in this cohort maintained features of differentiated leukocytes, which is important for AML classification and prognosis. Additionally, the DNA index had a mean of 1.84, reinforcing the genomic instability typically observed

in AML. Markers such as iMPO (mean = 43.99) and lysozyme (mean = 22) indicated the functional maturity of some leukemic cells, with these markers often being expressed in differentiated myeloid cells. Interestingly, the variability in the expression of these markers highlights the heterogeneity within leukemic blasts and could be correlated with different therapeutic responses and clinical outcomes. The variation in marker expression suggests the presence of distinct AML subtypes within the cohort, likely driven by different genetic and epigenetic abnormalities. For example, patients with t(15;17), corresponding to APL,

display markedly low CD34 expression (averaging 14.9%) and low HLA-DR (4.85%) expression, which is consistent with the characteristic differentiation block at the promyelocyte stage observed in APL. These patients also exhibit low CD11b expression, reflecting maturation arrest. The t(15;17) translocation is further notable for moderate to high iMPO expression (54.8%), indicative of enzymatic activity in immature myeloid cells. High CD33 expression (80.6%) reinforces the dominance of myeloid lineage commitment, further distinguishing t(15;17) as a distinct clinical and biological entity.

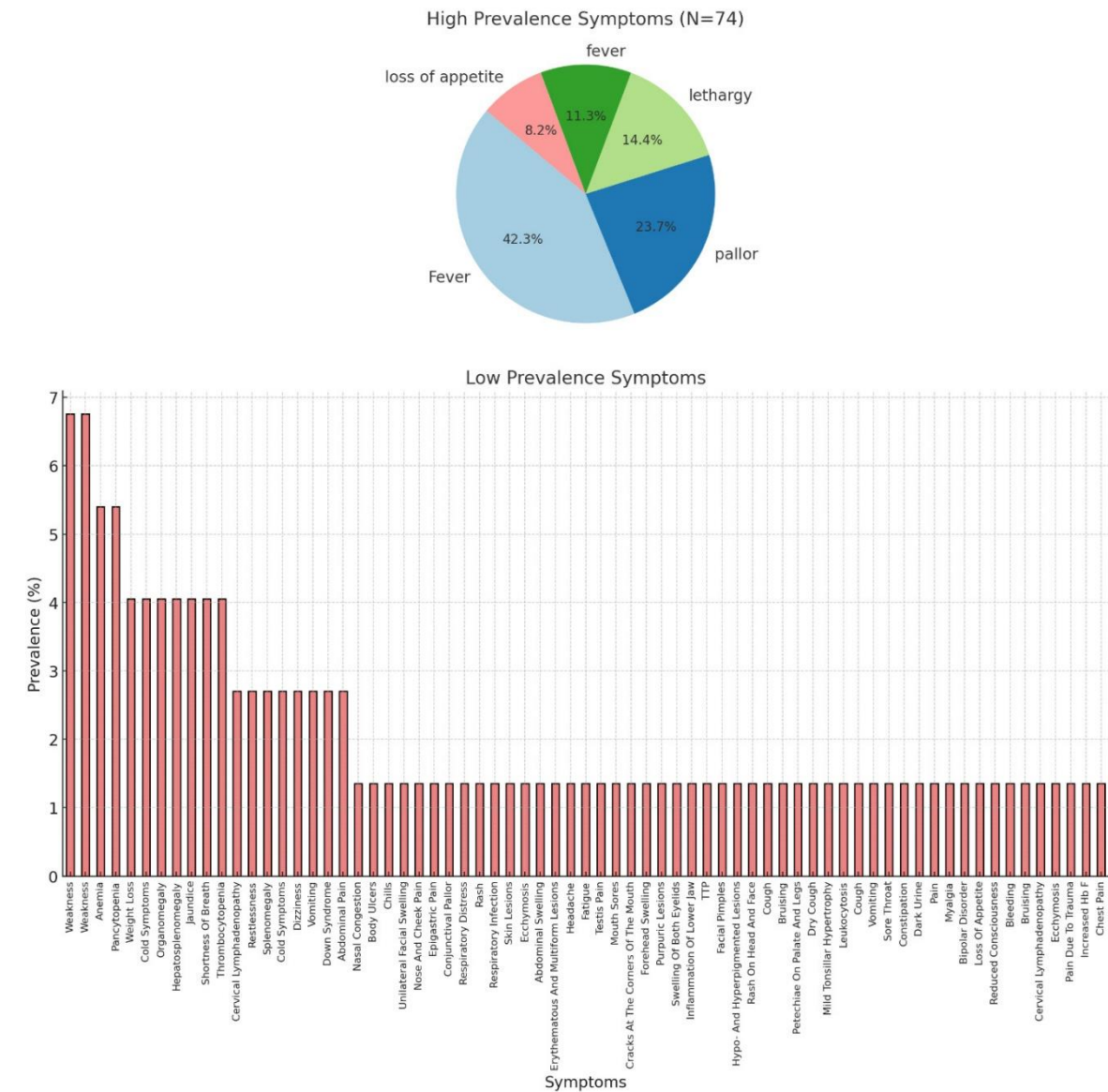


Figure 3. Distribution of symptom prevalence in the patient cohort (N = 74). The upper panel presents high-prevalence symptoms via a pie chart, depicting the relative proportions of common clinical features such as fever and pallor. The lower panel displays less frequent symptoms in a bar chart format, allowing for a more granular assessment of rarer clinical manifestations.

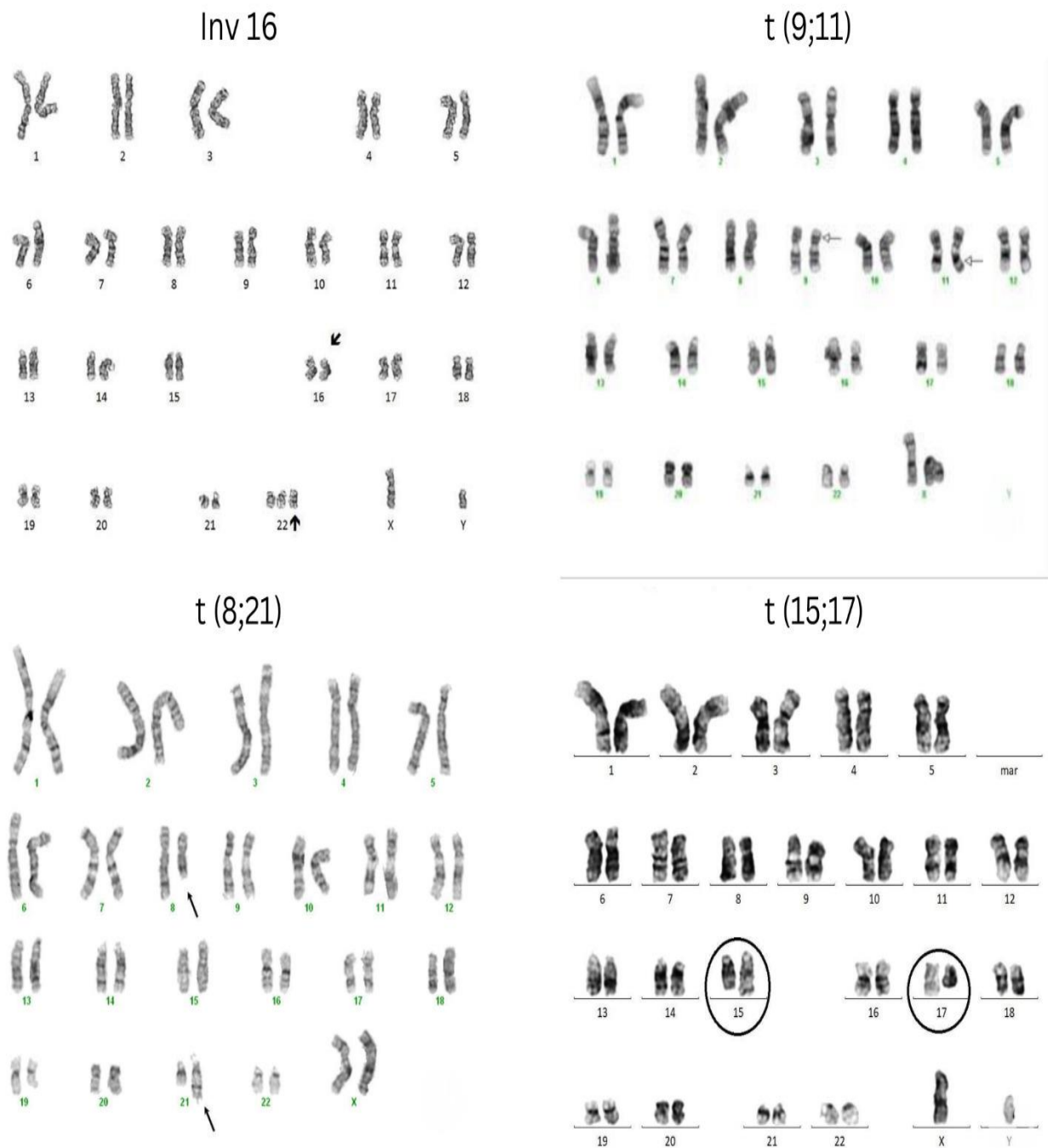


Figure 4. Karyotypic representation of key chromosomal translocations observed in AML. The figure depicts (a) inv(16)(p13q22), resulting in the CBFB–MYH11 fusion; (b) t(9;11)(p22;q23), associated with the KMT2A–MLLT3 fusion; (c) t(15;17)(q24;q21), producing the PML–RARA fusion characteristic of APL; and (d) t(8;21)(q22;q22), generating the RUNX1–RUNX1T1 fusion.

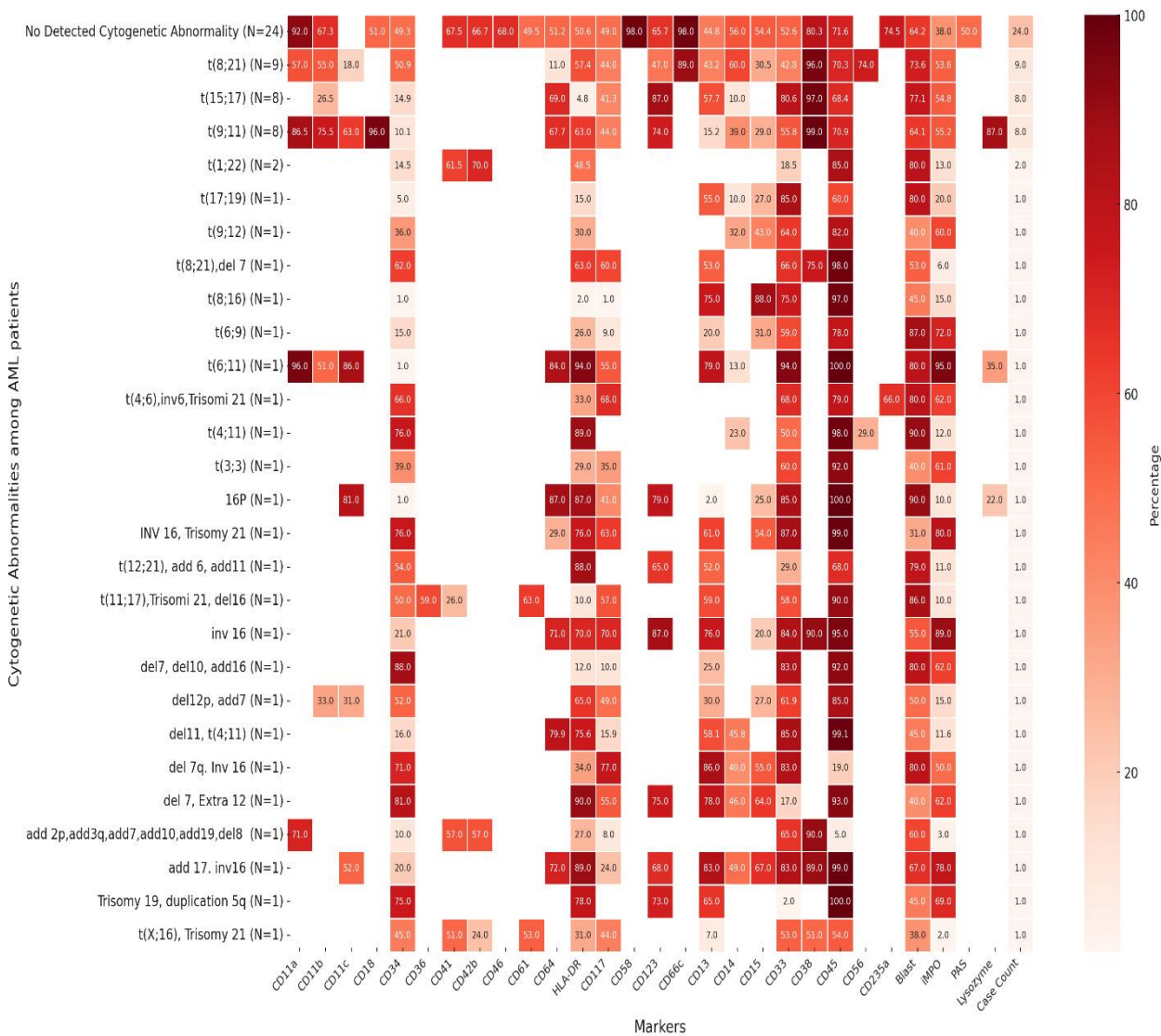


Figure 5. Heatmap showing average marker intensities across AML subtypes (N = 74), categorized according to cytogenetic abnormalities. The number of patients within each cytogenetic group is indicated in parentheses. Marker intensities are presented as the mean values for each subgroup, offering a comprehensive visualization of immunophenotypic profiles and highlighting potential differences in marker expression across distinct cytogenetic categories.

In contrast, patients with t(8;21), a translocation frequently associated with favorable prognosis in AML, presented higher CD34 expression (50.9%) and moderate CD33 expression (42.8%), reflecting a relatively preserved population of immature myeloid progenitors with partial myeloid maturation. The iMPO expression in this group was also moderate (53.6%), which is consistent with the typical morphology and maturation associated with this subtype. The consistently high CD45 expression (70.3%) across these patients reflects robust lymphohaematopoietic activity, aligning with the

generally favorable prognosis seen in t(8;21)-positive AML. Patients with t(9;11), often linked to monocytic differentiation, present with low CD34 expression (10.05%) and moderate CD33 expression (55.8%), indicative of a shift toward more differentiated monocytic cells. The iMPO expression (55.3%) in this group supports this differentiation trend, as iMPO is often expressed in less mature myeloid cells. These patients also exhibited moderate CD45 expression (70.9%), which may reflect the balance between immature and mature cell populations typical of this translocation. Additionally, patients with the rare

translocation t (6;11) show unique patterns, with extremely high expression of CD33 (94%) and CD45 (100%), as well as notable iMPO positivity (95%), distinguishing this subgroup by their heightened myeloid lineage commitment.

In patients with complex chromosomal alterations such as add (2p), add (3q), and del (8), the data demonstrate lower CD34 expression (10%) but a substantial reduction in CD45 (5%) and iMPO (3%), indicative of a diminished blast proliferation capacity and lower myeloid differentiation. Moreover, patients with del(7), Extra12 and del(7q), and inv(16) mutations presented high CD34 levels (81% and 71%, respectively) and moderate CD33 expression, which was consistent with preserved progenitor cell activity despite chromosomal deletions.

In terms of clinical relevance, the expression levels of markers such as CD45, CD33, and HLA-DR are often used to assess the differentiation stage of leukemic blasts and to predict the response to treatment. The DNA index and iMPO markers provide further understanding of the cellular environment, including chromosomal instability and the activation status of myeloperoxidase, respectively. These molecular features could be valuable in stratifying patients according to risk and guiding treatment strategies.

Discussion

This study provides a comprehensive analysis of the clinical, cytogenetic, and immunophenotypic characteristics of 74 pediatric and adolescent AML patients, revealing distinct patterns that correlate with underlying genetic alterations. Our findings reinforce the prognostic significance of specific mutations and chromosomal abnormalities in informing clinical presentation, risk stratification, and treatment strategies.

Elevated white blood cell counts were observed in patients with t(9;11), which aligns with previous reports that associated this translocation with hyperleukocytosis and an aggressive clinical course. Fatigue is a universal symptom in patients with inv (16), likely reflecting a high tumor burden. Immunophenotypic profiling revealed low CD34 and HLA-DR expression at t(15;17), which is consistent with the immunological signature of APL. These observations highlight the diagnostic value of

integrating cytogenetic and immunophenotypic data early in the disease course.

Evolving classification systems, such as the 2022 WHO (WHO5) and ICC, underscore the shift toward genomics-based AML subtyping. These systems now prioritize recurrent molecular lesions over purely morphological features. For example, AML with CEBPA mutations is now classified on the basis of specific in-frame bZIP domain mutations and is associated with a favorable prognosis, even in monoallelic cases, as long as the bZIP domain is involved. Similarly, TP53-mutated AML, newly designated as a distinct category of ICC, is recognized as a high-risk entity characterized by resistance to conventional therapy and poor overall survival (3, 4). These classifications support precision medicine approaches by identifying high-risk subgroups early and facilitating risk-adapted therapies.

Cytogenetic profiling remains a cornerstone of AML risk assessment. Our study revealed a high prevalence of translocations such as t(8;21), t(15;17), and t(9;11), each of which are associated with distinct clinical behaviors. The t(8;21) translocation, linked to the RUNX1-RUNX1T1 fusion, generally corresponds to the M2 AML subtype and is associated with favorable outcomes when treated with anthracycline-based regimens (4).

In contrast, t(15;17) defines APL and is characterized by the PML-RARA fusion, with high responsiveness to ATRA and arsenic trioxide, resulting in survival rates above 90% (19). In contrast, t(9;11), which frequently involves KMT2A (MLL) gene rearrangement, is associated with more aggressive disease and poorer outcomes. This rearrangement commonly occurs in infants and younger children and typically requires treatment intensification because of its intermediate to adverse prognostic implications (20).

In alignment with the 2022 European LeukemiaNet (ELN) recommendations, our genetic findings correspond with the updated risk stratification model used in clinical practice. For example, t(8;21) and inv(16) are classified under favorable risk, supporting standard induction–consolidation strategies. In contrast, patients with TP53 mutations, complex karyotypes, and KMT2A rearrangements such as t(9;11) fall under the adverse risk group, indicating the need for intensified or alternative therapies and early transplant evaluation. The 2022

ELN guidelines emphasize the role of integrated molecular and cytogenetic profiling in risk-adapted management, reinforcing the clinical relevance of our study's findings (21). The clinical manifestations of AML vary widely on the basis of genetic subtype. Patients with t(15;17) commonly present with bleeding and bruising due to coagulopathies and DIC (22, 23). In contrast, those with trisomy 19 or duplication 5q had fewer systemic symptoms, suggesting a less aggressive disease course (24). Fatigue is universally reported in inv(16), which is consistent with its association with high tumor burden.

Hematologic profiles also vary: hyperleukocytosis at t(9;11) and thrombocytopenia at t(15;17) support their respective aggressive and hemorrhagic tendencies (25-27). Rare abnormalities, including t(1;22) and inv(16), were also observed. Although infrequent, their recognition is critical given their prognostic implications and potential to cooccur with mutations such as FLT3-ITD or KIT (16, 28-33).

Immunophenotypic analysis further supported the diagnostic and prognostic stratification of AML subtypes. Consistent with prior studies, high expression of CD34 and CD33 was observed in the majority of cases, indicating immature myeloid phenotypes and supporting the rationale for CD33-targeted therapies such as gemtuzumab ozogamicin (34)(12). Variation in CD45 and HLA-DR expression provides additional insight into leukemic differentiation and immune evasion. Notably, low HLA-DR expression was particularly evident at t(15;17), mirroring the classical immunophenotype of APL (35). The findings of this study reinforce the need for individualized treatment strategies informed by genetic and immunophenotypic profiles. Early recognition of APL-associated cytogenetics allows for the immediate initiation of differentiation therapy, reducing mortality from DIC. The aggressive phenotype associated with t(9;11) supports early treatment intensification and consideration of investigational agents (36, 37).

Our findings also support the integration of NGS into routine clinical workflows. The availability of targeted therapies—including FLT3 inhibitors (e.g., midostaurin), IDH1/2 inhibitors (ivosidenib, enasidenib), and BCL-2 inhibitors (venetoclax)—has enabled a more personalized approach to therapy that is matched to specific genomic alterations (38). As NGS becomes more accessible, its incorporation will

further refine risk stratification and therapeutic selection.

Finally, the future of AML therapy lies in personalized, genomically informed treatment paradigms. Trials such as myeloMATCH and ImmunoMATCH represent efforts to align therapy selection with the patient's molecular and immunologic profile, ensuring maximal therapeutic efficacy with minimal toxicity (39, 40). These efforts reflect a broader move toward precision oncology, in which therapies are selected not only on the basis of disease subtype but also on the basis of individual genetic and immunological profiles. Our study supports this approach and highlights the value of integrating cytogenetic data into real-time clinical decision-making (41).

Despite the valuable insights provided by our study, we acknowledge its limitations, including its retrospective design and single-center cohort, which may limit the generalizability of the findings. Future studies should involve larger, multicenter datasets and utilize comprehensive molecular platforms, including NGS, to validate these results and refine the prognostic models used in pediatric AML patients (9).

This study provides comprehensive insights into the clinical and genetic landscape of pediatric AML. By elucidating the correlation between cytogenetic abnormalities and clinical outcomes, our findings emphasize the importance of integrating genetic profiling into diagnostic and therapeutic strategies. This approach not only enhances risk stratification but also paves the way for more personalized treatment regimens, ultimately improving survival rates and quality of life for patients. The heterogeneity of AML underscores the need for continuous research into novel therapeutic modalities and the integration of advanced molecular diagnostics to refine our understanding of the disease. Future studies should focus on multicenter data and NGS data to further explore the genetic underpinnings and therapeutic implications of AML.

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