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*Original Article*

# A comprehensive analysis of cytogenetics, molecular profile, and survival among pediatric acute myeloid leukemia: a prospective study from a tertiary referral center

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**Abstract:** Background and aims: The objectives of this study were to investigate the cyto-molecular profile and survival of pediatric acute myeloid leukemia (AML). Methods: This prospective study was carried out in a tertiary care hospital from October 2018 to December 2020. Karyotype and cytogenetics analyses were done to identify chromosomal aberrations in pediatric AML. The targeted molecular panel utilized the polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), and fragment analysis. Results: A total of 70 patients of AML with aged ≤18 years were enrolled in this study. The cytogenetic analyses revealed abnormal/recurrent cytogenetic abnormalities (CA) in 64.3% of patients and normal cytogenetics (CN) in 35.7% of patients. FAB M2 subtype showed frequent aberrant expression of the CD19 marker. CD7, CD11b, and CD36a were significantly present in the absence of molecular markers. Common chromosomal abnormalities were t(translocation) (8;21) (55%), monosomy/deletion 7 (13%), monosomal karyotype (5%) and complex karyotype (3%). The fusion transcripts *RUNX1-RUNX1T1* [t(8;21)] (41%) and *CBFB-MYH11* [t(16;16)] (3%) were detected by RT-PCR and FLT3-TKD D835 mutation (1.5%) by allele-specific oligo PCR. Fragment analysis revealed NPM1 (8%) mutation and FLT-ITD (9.5%) mutations. Complete remission was achieved in all evaluable patients. The median follow-up period of our patients was 225 days (IQR 28; 426 days). The median event-free survival (EFS) in all patients was 11.9 months (95% CI, 5-12.6 months). The forty months overall survival probability (pOS) was 58% in all patients. Conclusion: The majority of patients had abnormal/recurrent cytogenetics abnormalities. FAB M2 subtype showed frequent aberrant expression of the CD19. The absence of molecular markers may suggest the presence of CD7, CD11b, and CD36a expression. The overall survival has increased considerably in LMIC.

**Keywords:** Acute myeloid leukemia, karyotype, cytogenetics, molecular, children, survival

## Introduction

AML is a heterogeneous hematologic malignancy characterized by clonal expansion of myeloid blasts in the bone marrow, peripheral blood, and other tissues. It has varied presentations and is 2nd most common childhood leukemia after acute lymphoblastic leukemia (ALL). Pediatric AML comprises 15-20% of leukemia, with an incidence of seven cases per million in <15 years of age [1]. AML blasts are malignant myeloid progenitor cells that fail to differentiate, proliferating in the bone marrow and invading peripheral blood and other organs, such as the central nervous system. Pediatric AML has better survival than adults because of the more frequent presence of good prognostic genetic features and higher tolerance to intensive treatment in children [2].

The treatment outcome of AML depends on the initial response to therapy and molecular and cytogenetic aberrations. Chromosomal abnormalities are recognized as important diagnostic and prognostic indicators [3]. The most frequent cytogenetic abnormalities are balanced

chromosomal rearrangements in pediatric AML. Unbalanced chromosomal abnormalities, such as monosomy 5 and 7, are less frequent in children and are associated with poor outcomes [3]. The cytogenetic and molecular abnormalities are involved in the pathogenesis of AML, and clonal chromosomal abnormalities are found in 70-85% of pediatric AML [4]. Several cyto-molecular events define the distinct subtypes of AML in childhood. These changes can be used as markers and help better define therapy targets, thereby reducing the toxicity of current treatment strategies [5].

The immunophenotypic features, molecular abnormalities, and recurrent mutations observed in AML provide potent markers for detecting measurable or minimal residual disease (MRD), which is an important prognostic marker in the treatment of AML [6].

The prognosis for children with AML has significantly improved over the last three decades due to the advancements in diagnostic technology, hematopoietic stem cell transplant (HSCT), and the introduction of newer chemotherapeutic agents. However, the overall survival of AML remains <70% [5]. The scenario of AML treatment is different in low-middle-income countries (LMIC), like India, where resources are limited and adequate supportive care facilities are not universally available in all centers [7]. A few families of children with AML do not opt for treatment, and many patients die of treatment-related toxicity. There are limited data available in the literature on AML from LMIC regarding the cyto-molecular profile and treatment outcome. Hence, this study aimed to evaluate the cyto-molecular analyses and outcomes of pediatric AML in India.

## Materials and methods

### *Study design and patients*

This prospective study was conducted from October 2018 to December 2020 at the Department of Pediatrics, Department of Medical Oncology, and Laboratory Oncology Unit in All India Institute of Medical Sciences (AIIMS), New Delhi, India. We obtained approval from the Institute Ethics Committee at AIIMS (IEC-383/06.07.2018, RP-7/2018) for this study. Newly diagnosed patients with AML were eligible for enrolment if they were ≤18 years of age and parents or legally authorized representatives (LAR) signed the informed consent. The exclusion criteria included mixed phenotyping acute leukemia (MPAL), secondary leukemia, AML with Down syndrome, and acute promyelocytic leukemia (APML). In all patients, karyotype/cytogenetics analyses were done to identify chromosomal aberrations, and polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), and fragment analysis were utilized for the targeted molecular panel.

The demographics, clinical characteristics, laboratory parameters, and survival were compared between the two groups [abnormal cytogenetics/recurrent cytogenetic abnormalities AML patients (CA-AML) *vs* normal cytogenetics AML patients (CN-AML)]. The demographic profile and clinical and laboratory parameter assessments included the following:

Baseline demographics: We recorded the age, gender, anthropometry, nutrition status, and education status of the parents. The anthropometry and nutritional status were assessed after the completion of each chemotherapy cycle and every three months after the completion of treatment for two years, and, after that, every six months.

Clinical profile: We collected data on patients' symptoms duration, history of fever, bleeding, pallor, proptosis, fatigue, loss of appetite, abdominal distension, loss of weight, and joint pain. We also recorded the presence of extramedullary disease. We looked for splenomegaly, hepatomegaly, and lymphadenopathy. The presence or absence of organomegaly was assessed after completion of each chemotherapy course, every three months post-completion of treatment for two years, and after that, every six months. These parameters were evaluated by a detailed history and physical examination at each visit.

Laboratory characteristics: Patients' baseline complete blood counts (CBCs) were recorded [Hb (gm/dl), TLC (/mm$^3$), ANC (/mm$^3$), and platelets (/mm$^3$)]. A ten-color multiparameter flow cytometer processed samples for immunophenotyping using bulk lyse and staining methods. The cell suspension from the sample was prepared by bulk erythrocyte lysing with ammonium chloride-based lysing reagent. After lysis

and wash, the remaining cells were resuspended in phosphate-buffered saline (PBS) with 5% bovine serum albumin (BSA). The cells were then stained for immunophenotyping using 10-color antibody panels. All cells were fixed with 0.5% paraformaldehyde and stored at 4°C. The analysis was done within 6 hours of staining. Samples were acquired on a three-laser Gallios flow cytometry instrument (Beckman Coulter, BC). For the diagnostic immunophenotyping, 25,000 to 50,000 events per tube were acquired. Immunophenotyping data were analyzed with Kaluza (version 1.3) software (Beckman Coulter, USA). The presence of MPO-positive blasts and CD markers was recorded.

CBCs were checked weekly and as per clinical indication and before starting a new chemotherapy cycle during the treatment. CBCs and peripheral smear examinations were assessed every three months after completion of therapy for two years and, after that, every six months.

The bone marrow flow cytometry was performed to check remission status post-induction-1, and if the bone marrow was not in remission, then it was repeated post-induction-2. When there was clinical suspicion, and CBCs/peripheral smear suggested relapse, flow cytometry was again performed to confirm the relapse.

*Outcome assessment*

The primary objectives of this study were to investigate the cyto-molecular profile, and the secondary objective was to determine the survival of pediatric AML.

*Sample collection and processing*

In suspected cases of acute myeloid leukemia, samples were collected during bone marrow procedures or from the peripheral blood where the blasts counts were at least 40%, and total leukocyte counts (TLCs) were normal to high. Peripheral blood (4 ml)/bone marrow sample (2 ml) was collected in sodium heparin (for karyotype/cytogenetics) and ethylenediaminetetraacetic acid (EDTA) tubes (for PCR, RT-PCR, and fragment analysis).

The diagnosis of AML was established as per WHO criteria by demonstrating the involvement of more than 20% of leukemic myeloblasts in peripheral blood (PB) and/or bone marrow (BM) [3]. The diagnosis was further confirmed by flow cytometry. The karyotype/cytogenetics of all patients were analyzed using conventional karyotyping. Allele-specific oligo PCR was used for FLT3-TKD (D835Y) mutation detection, while NPM1 mutation and FLT3-ITD mutations were detected using fragment analysis in all the samples. All confirmed cases of AML were enrolled in this study, and the appropriate treatment protocol was assigned.

*Molecular testing*

Fusion transcripts AML-ETO t(8;21), and CBFB-MYH11 t(16;16)/inv(16) were detected using previously described RT-PCR protocol [8]. For fragment analysis, one-step multiplex PCR was performed for simultaneous detection of FLT3/ITD and NPM1 mutations [9]. Primer sets were previously described to detect the presence or absence of FLT3/ITD and NPM1 4 bp insertion mutations. The multiplex PCR reaction contained 100 ng DNA, 0.4 μM of both primer sets, 1.25 mM $MgCl_2$, 0.25 mM dNTP, 2.5 μl 10 × PCR buffer, 0.2 μl Taq DNA polymerase, and water in 25 μl total volume. The PCR conditions were: initial denaturing for 5 min at 95°C, followed by 35 cycles at 95°C for the 30 s, 59°C for 40 s, and 72°C for the 40 s with a final cycle of 10 mins at 72°C. The PCR products were then diluted at 1:100 and analyzed by capillary electrophoresis on ABI 3500 Genetic Analyzer according to the manufacturer's protocol. A 330 bp fragment corresponded to wild-type FLT3, and 170 bp was detected for NPM1 wild-type.

Allele-specific oligo PCR was used for FLT3-TKD (D835Y) mutation detection. PCR amplification was as described above using specific primers for exon 20 (5'-CCGCCAGGAACGTGCTTG-3' and 5'-GCAGCCTCACATTGCCCC-3'). PCR product was digested with EcoRV (NEB) at 37°C for two hours [10]. The digestion products were separated on a 3% agarose gel, and incomplete digestion indicated the presence of a mutant.

*Treatment plan*

Patients with AML treated with either ADE (cytosine arabinoside-10 + daunorubicin-3 + etoposide-5), or DA (cytosine arabinoside-7 + daunorubicin-3) or cytosine arabinoside-10 + daunorubicin-3 for induction.

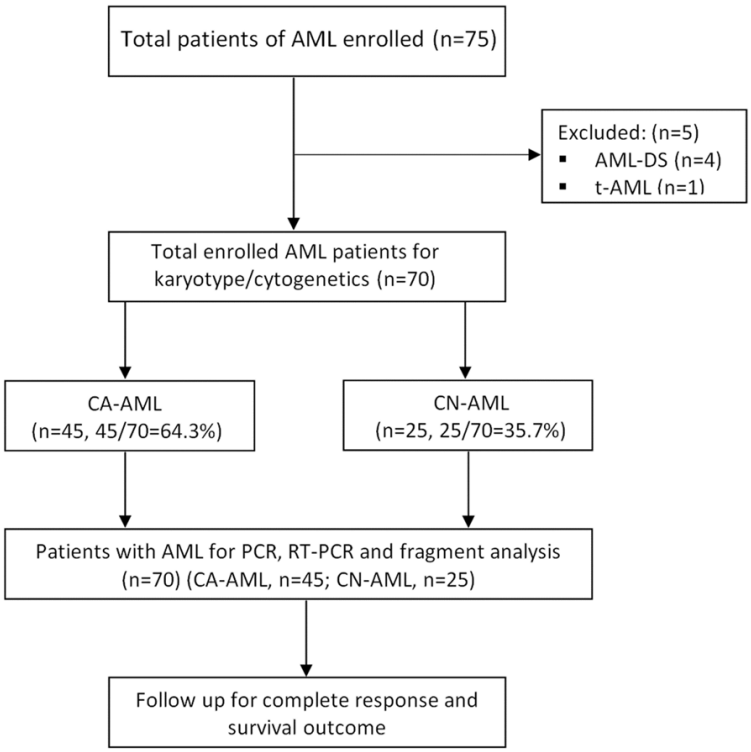


**Figure 1.** Flow diagram of study (AML-DS: acute myeloid leukemia with Down syndrome; t-AML: therapy-related acute myeloid leukemia; CA-AML: AML with abnormal cytogenetics; CN-AML: AML with normal cytogenetics; RT-PCR: Reverse transcription-polymerase chain reaction).

*Evaluation and follow-up of patients*

All evaluable patients underwent post-induction bone marrow for the assessment of morphological remission. Patients were evaluated for event-free survival (EFS) and overall survival (OS). The data were censored on 30$^{th}$ June 2022 by ascertaining the final condition by clinical and laboratory evaluation or telephonic follow-up. The indicators used to determine outcomes were time to relapse, time to death, and most recent follow-up. The cause of death was classified as either related to or unrelated to the disease.

*Definition*

Monosomal karyotype (MKs) is the co-occurrence of two or more autosomal monosomies or one autosomal monosomy with at least one structural abnormality (excluding marker or ring chromosomes) and without favorable chromosomal abnormalities [1].

Complex karyotype refers to ≥3 abnormalities, including one structural aberration, excluding favorable cytogenetics and KMT2Ar [3].

Complete remission (CR) was defined as the bone marrow regenerating normal hematopoietic cells and contained <5% blast cells by morphology after induction.

Event-free survival (EFS) was determined as the time from the diagnosis of AML to relapse, death, or the patient's most recent follow-up.

Overall survival (OS) was calculated as the time from diagnosis to death or the patient's most recent follow-up.

*Statistical methods*

Descriptive statistics such as proportions, mean (± SD), and median (IQR) were used to describe all patients' baseline demographics, clinical profiles, and laboratory characteristics. A chi-square test/Fisher exact test was used to determine the association between two categorical variables. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was applied to compare two continuous variables. The survival analyses [event-free survival (EFS) and overall survival (OS)] were depicted using Kaplan Meier plots/log-rank test. The Cox proportional hazard model was also used to identify the probability of events between two groups. Data were censored on 30$^{th}$ June 2022. The *p*-value <0.05 was considered a significant level. All the data analyses were performed using STATA 14.

## Results

*Patients recruitment and baseline characteristics*

A final diagnosis of AML was made in 75 children aged ≤18 years. Further five patients were excluded [AML with Down syndrome (AML-DS) (n=4), therapy-related AML (t-AML) (n=1)]. Finally, 70 patients with AML underwent conventional karyotyping/cytogenetic analyses, and PCR, RT-PCR, and fragment analyses were

**Table 1.** Demographics and clinical characteristics of all enrolled acute myeloid leukemia (AML) patients (n=70), AML with abnormal/recurrent cytogenetic abnormalities (CA-AML) (n=45) and AML with normal cytogenetics (CN-AML) (n=25)

| Baseline characteristics | n=70 (%) | n=45 (%) | n=25 (%) | *p*-value |
|---|---|---|---|---|
| Median age (years) (IQR) | 6 (3; 11) | 6 (3; 10) | 7 (4.5; 11) | 0.77 |
| Male:Female | 3:2 | 5:4 | 2.1:1 | 0.30 |
| Median weight (IQR) | 17.65 (12.8; 24) | 17 (13; 23) | 18 (12.8; 24) | 0.97 |
| Mean height (± SD) | 117.7 (25.9) | 111.5 (25.7) | 115 (26.5) | 0.76 |
| Malnutrition | | | | 0.53 |
| Present | 17 (24%) | 12 (27%) | 5 (20%) | |
| Absent | 53 (76%) | 33 (73%) | 20 (80%) | |
| Guardians' education status | | | | 0.35 |
| Illiterate | 9 (13%) | 7 (16%) | 2 (8%) | |
| Upto class 12$^{th}$ | 45 (65%) | 26 (59%) | 19 (76%) | |
| Graduation and above | 15 (22%) | 11 (25%) | 4 (16%) | |
| Clinical presentation | | | | |
| Median duration of symptoms (days) (IQR) | 25 (10; 45) | 20 (10; 45) | 30 (15; 45) | 0.43 |
| Fever | 58 (83%) | 39 (86.7%) | 19 (76%) | 0.25 |
| Bleeding | 19 (27.5%) | 12 (27%) | 7 (28%) | 0.94 |
| Pallor | 36 (51.4%) | 25 (55.6%) | 11 (44%) | 0.35 |
| Proptosis | 13 (18.6%) | 9 (20%) | 3 (12%) | 0.25 |
| Fatigue | 12 (17%) | 6 (13%) | 6 (24%) | 0.25 |
| Loss of appetite | 10 (14.3%) | 4 (9%) | 6 (24%) | 0.08 |
| Abdominal distension | 9 (12.9%) | 6 (13%) | 3 (12%) | 0.87 |
| Loss of weight | 8 (11.4%) | 5 (11%) | 3 (12%) | 0.91 |
| Joint pain | 3 (4.3%) | 0 | 3 (12%) | 0.04 |
| Extramedullary disease | 17 (24.3% | 12 (27%) | 5 (20%) | 0.67 |
| Eye | 13 (18.6%) | | | |
| Intracranial, sinonasal, mastoid, ear, elbow, thigh | 8 (11.4%) | | | |
| Physical examination | | | | |
| Hepatomegaly | 49 (70%) | 32 (71%) | 17 (68%) | 0.78 |
| Splenomegaly | 31 (44.3%) | 21 (46.7%) | 10 (40%) | 0.59 |
| LAP | 19 (35.9%) | 11 (33%) | 8 (32%) | 0.62 |
| Median size of liver below costal margin (IQR) | 2 (0; 3.5) | 2 (0; 4) | 2 (0; 3) | |
| Median size of spleen below costal margin (IQR) | 0 (0; 2) | 0 (0; 2) | 0 (0; 2) | |
| Median size of LAP (IQR) | 0 (0; 2) | 0 (0; 1.5) | 0 (0; 2) | |

TLC: total leucocyte counts; ANC: absolute neutrophil counts; ALC: absolute lymphocyte counts; LAP: Lymphadenopathy.

utilized for the targeted molecular panel (**Figure 1**).

The median age of this cohort was six years (IQR; 3, 11 years), and males were more in number (M:F: 3:2). About one-fourth of patients had malnutrition [11, 12]. The median duration of disease symptoms before presentation to the hospital was 25 days (IQR; 10, 45 days). Fever was the most common symptom, followed by bleeding, pallor, proptosis, and fatigue. Approximately one-fourth of patients had an extramedullary disease. Hepatomegaly, splenomegaly, and lymphadenopathy were seen in 70%, 44.3%, and 35.9% of patients. The demographics and clinical characteristics were similar between the two groups (CA-AML *vs* CN-AML) except for joint pain, which was observed only in CN-AML patients (*P*=0.04) (**Table 1**).

The baseline mean hemoglobin (Hb) was 6.7 gm dl (SD ± 2.5). The median total leukocyte counts (TLCs), median absolute neutrophil

**Table 2.** Laboratory characteristics of all AML patients (n=70), AML with abnormal/recurrent cytogenetic abnormalities (CA-AML) (n=45) and AML with normal cytogenetics (CN-AML) (n=25)

| Laboratory characteristics | n=70 (%) | n=45 (%) | n=25 (%) | *p*-value |
|---|---|---|---|---|
| Mean Hb (gm/dl) (SD) | 6.7 (± 2.5) | 6.6 (2.4) | 6.9 (2.7) | 0.69 |
| Median TLC (/mm$^3$) (IQR) | 25395 (10970; 92850) | 22770 (11680; 75740) | 49850 (10460; 130000) | 0.42 |
| Median ANC (/mm$^3$) (IQR) | 3040 (1570; 10400) | 3230 (1750; 9250) | 2200 (970; 12060) | 0.56 |
| Median platelets (/mm$^3$) (IQR) | 32000 (15000; 70000) | 33000 (14000; 64000) | 31000 (17000; 103000) | 0.23 |
| Presence of CD markers in flowcytometry | | | | |
| CD33 | 64 (94%) | 41 (93.2%) | 23 (92%) | 0.65 |
| CD13 | 62 (91%) | 42 (95.5%) | 20 (80%) | 0.09% |
| CD38 | 57 (83.8%) | 39 (88.6%) | 18 (72%) | 0.14 |
| CD117 | 52 (76.5%) | 36 (81.8%) | 16 (64%) | 0.15 |
| CD34 | 51 (75%) | 34 (77.3%) | 17 (68%) | 0.55 |
| CD45 | 33 (48.5%) | 20 (45.5%) | 13 (52%) | 0.49 |
| CD56 | 22 (32.4%) | 16 (36.4%) | 6 (24%) | 0.33 |
| CD11b | 21 (30.9%) | 14 (31.8%) | 7 (28%) | 0.82 |
| CD7 | 18 (26.5%) | 10 (22.7%) | 8 (32%) | 0.34 |
| CD123 | 19 (27.9%) | 12 (27.3%) | 7 (28%) | 0.86 |
| CD64 | 15 (22%) | 8 (18.2%) | 7 (28%) | 0.29 |
| CD19 | 11 (16%) | 11 (25%) | 0 | 0.006 |
| CD36 | 11 (16%) | 7 (15.9%) | 4 (16%) | 1.00 |
| CD41 | 2 (2.9%) | 2 (2.6%) | 0 | 0.53 |
| CD71 | 2 (2.9%) | 1 (2.3) | 1 (4%) | 0.65 |
| FAB classification | | | | |
| M0 | 1 (1.5%) | 1 (2.3%) | 0 | 0.02 |
| M1 | 4 (6%) | 0 | 2 (8%) | |
| M2 | 36 (55.4%) | 28 (65%) | 8 (32%) | |
| M4 | 15 (21.4%) | 11 (25.6%) | 6 (24%) | |
| M5 | 5 (7%) | 2 (6.7%) | 4 (16%) | |
| M7 | 1 (1.5%) | 1 (2.3%) | 2 (8%) | |
| UC | 5 (7%) | 2 (6.7%) | 3 (12%) | |
| MPO positive blasts | 48 (69%) | 31 (68.9%) | 17 (68%) | 0.93 |
| CNS involvement | 3 (4.3%) | 1 (2.3%) | 2 (8%) | 0.25 |

Hb: Hemoglobin; TLC: total leucocyte counts; ANC: absolute neutrophil counts; MPO: Myeloperoxidase; CNS: Central nervous system.

counts (ANC), and median platelets counts were 25395/mm$^3$ (IQR; 10970, 92850), 3040/mm$^3$ (IQR; 1570, 10400), and 32000/mm$^3$ (IQR; 15000, 70000), respectively. The CD markers which were present in more than 50% of patients were CD33 (94%), CD13 (91%), CD38 (83.8%), CD117 (76.5%) and CD34 (75%). Most patients were the AML subtypes of M2 (55.4%) and M4 (21.4%). The myeloperoxidase (MPO) was positive in 69% of blast cells, and 4% of patients had CNS positivity at baseline. The CD19 marker was detected only in CN-AML patients (*P*=0.006) (**Table 2**).

*Karyotype/cytogenetics results*

Karyotype/cytogenetics was analyzed in all patients (n=70). Out of 70 patients, abnormal/recurrent cytogenetic abnormalities (CA) were observed in 45 (64.3%) patients and normal cytogenetics (CN) in 25 (35.7%) patients. Amongst the CA-AML group, the commonest abnormality was t(8;21) (55%), which is classified under the standard risk [3]. The second most common abnormality observed was del/monosomy 7 (13%), which is classified under the adverse risk group [3]. Additionally, monosomal karyotype was observed in 5% and complex karyotype in 3% of patients. Other atypical karyotypes observed were add51, add21; del5; t(1;11); t(11;19); t(9;12). In this study, t(8;21) cytogenetics was co-incident with X0 karyotype. Trisomy 8 was present in two patients, and in one patient, it was co-incident with t(9;11) (**Figure 2A**). Of the patients (n=11)

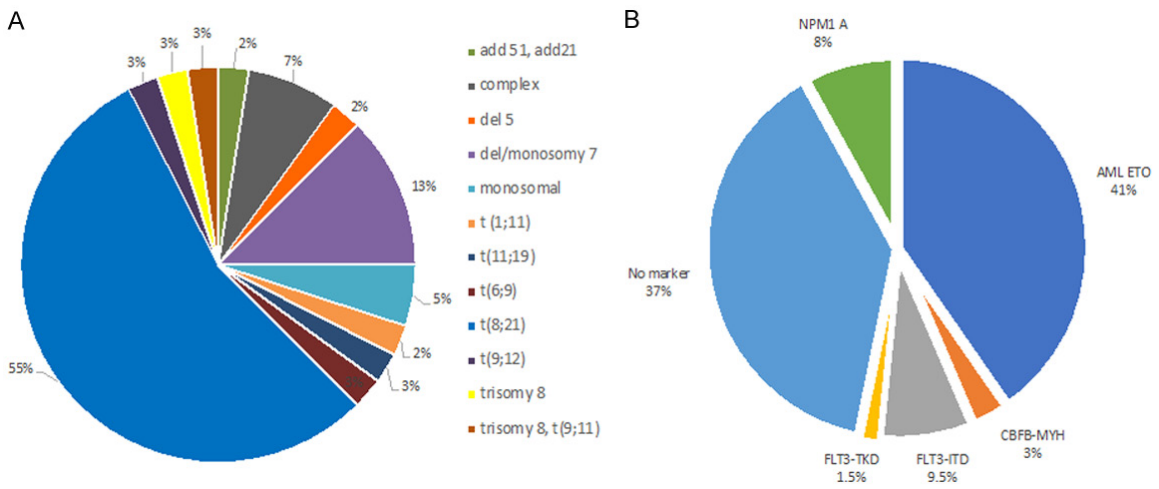


**Figure 2.** A. Abnormal/recurrent cytogenetic abnormalities (n=45) (CA-AML group), B. Molecular aberrations (n=70).

who were detected to have aberrant expression of CD19, seven patients had t(8;21), one patient had t(8;21) and del(9), and one had t(8;21), t(2;21), and der(21) on the cytogenetics and the samples of two patients had culture failure (Supplementary Table 1).

*Molecular panel results*

AML-ETO/t(8;21), BCR-ABL/t(9;22), CBFB-MYH11/t(16;16)/inv(16) are the commonly occurring translocations of AML and we used RT-PCR assay in all patients (n=70) to detect it. Out of 70 patients (both CA-AML and CN-AML groups), samples of 65 patients could be analyzed (as the blast cell inadequacy could not produce desirable results in five patients). Amongst the mutations targeted by the molecular panel, AML-ETO was the most frequent, with a presentation in 41% of patients. AML-ETO fusion co-occurred with FLT3-ITD mutation in two patients, and one patient harbored NPM1. FLT3-ITD mutations were observed in six (9.5%) patients. At the same time, NPM1 mutations were detected in 8% of patients. CBFB-MYH11 was detected in 3.2% and FLT3-TKD (835Y) in 1.5% of patients. In the CA-AML group, FLT3-ITD occurrence frequency was 9.5%, and for AML-ETO, it was 52.3% (**Figure 2B**). Of the CA-AML patients (n=11) who were detected to have aberrant expression of the CD19 marker, 9 had AML-ETO (82%), one had FLT3-ITD, and one had both AML-ETO and FLT3-ITD (Supplementary Table 1).

*Correlation between CD markers and cytomolecular analyses*

In this cohort, the aberrant expression of CD19 was correlated with the presence of cytogenetic abnormalities ($P$=0.01), and it was associated with the presence of t(8;21). CD7 ($P$=0.00), CD11b ($P$=0.01) and CD36a ($P$=0.00) were significantly present in the absence of molecular markers. The aberrant expression of CD19 ($P$=0.01) was significantly detected in the presence of molecular markers, and AML-ETO was observed in most of these patients (82%).

*The outcome of induction in all patients*

Out of 70 patients, for induction, 41 (59%) received ADE (cytosine arabinoside-10 + daunorubicin-3 + etoposide-5), 22 (38%) received DA (cytosine arabinoside-7 + daunorubicin-3) and 3 (4%) received cytosine arabinoside-10 + daunorubicin-3. The median duration from the diagnosis to treatment initiation was 4.5 days (IQR: 2; 8 days). One patient received only four doses of cytosine arabinoside, and families of three patients (4%) declined treatment. Complete remission (CR) was observed in all evaluable patients (100%). In this study, 26 (37%) patients died in a follow-up of 40 months, 21 (30%) patients died in induction (81% of total mortality), and among all mortality, 69% of deaths (n=18) were due to sepsis. About one-third (33%) of patients had a relapse in a follow-up period of 40 months.

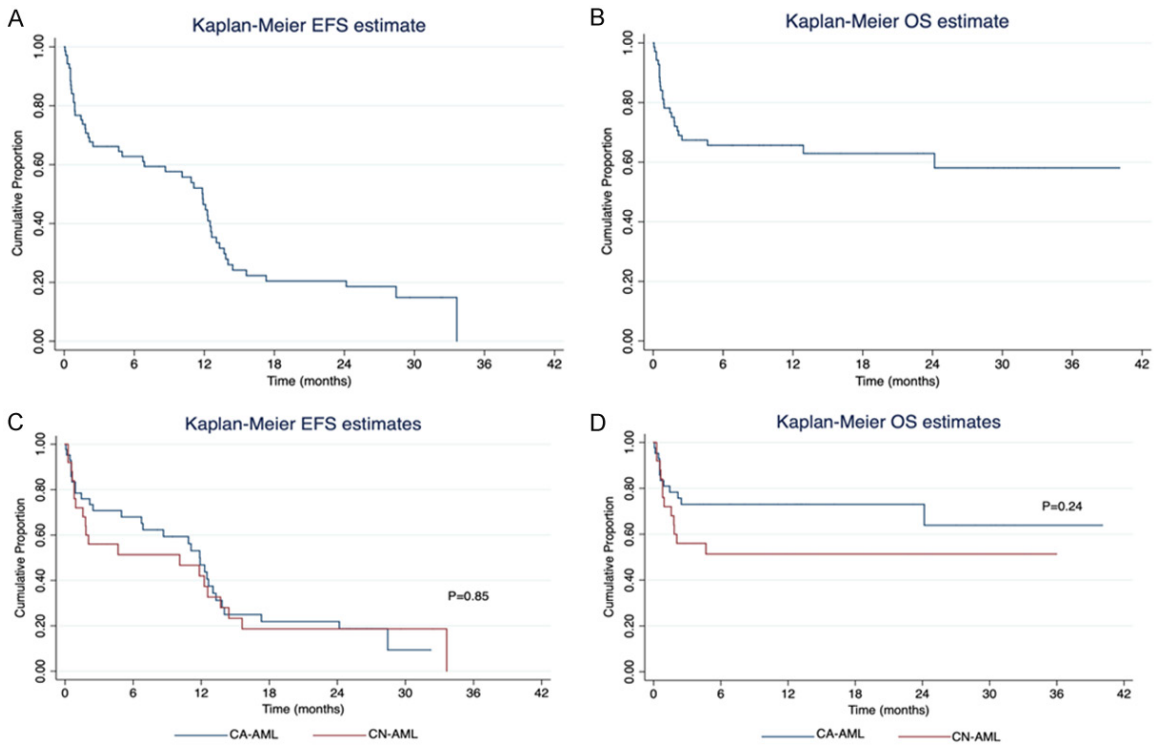


**Figure 3.** Event-free survival (EFS) and overall survival (OS) curves. A. Forty months EFS for all patients; B. 40 months OS for all patients; C. 40 months EFS by category; D. 40 months OS by category. (EFS: Event-free survival; OS: Overall survival; CA-AML: AML with abnormal/recurrent cytogenetics abnormalities; CN-AML: AML with normal cytogenetics).

*Survival outcome*

The median follow-up period of our patients was 225 days (IQR 28; 426 days). The median event-free survival (EFS) in all patients was 11.9 months (95% CI, 5-12.6). The forty months overall survival probability (pOS) was 58% in all patients. There was no significant difference in EFS and OS between CN-AML (pOS, 53%) vs CA-AML group (pOS, 63%) (*P*=0.24) [EFS: HR=1.05 (95% CI, 0.59-1.87); and OS: HR=1.58 (95% CI, 0.72-3.5)] (**Figure 3**).

## Discussion

Treatment of childhood malignancies has markedly improved over the past few decades, and the overall mortality rate has declined. Pediatric AML is a heterogeneous disease with generally poor outcomes compared to ALL. The role of advanced in-patient facilities and the latest techniques is indispensable in improving disease outcomes [13].

In this study, there were more male patients with AML (60%), consistent with the literature reporting a male predominance in the incidence of AML [14]. AML was diagnosed at a median age of 6 years, similar to the literature that reports a mean age of 6 years at diagnosis [1]. In the CCG 2691 study, Sung et al found that underweight status increased the risk of infection-related mortality [15], and in the index study, one-fourth of patients had malnutrition. The prevalence of extramedullary disease (EMD) was 28% (24% myeloid sarcoma; 4% CNS), similar to that reported in other studies [16, 17]. The prognosis in these cases of EMD is still controversial [18]. Some authors believe that EMD has a worse prognosis [19], while others argue that there is no relationship between EMD and the prognosis of the disease [20], and some authors reported that it has a good prognosis [21]. In our cohort, the median TLC count was 25395/mm$^3$. An initial high TLC count (>100 × mm$^3$) is generally associated with a worse prognosis [22]. The FAB M2 (55%) subtype was the highest, followed by the FAB M4 (21%) [23]. The aberrant expression of CD19 was found only in the CA-AML group. Recently a meta-analysis reported that the

aberrant expression of the CD19 marker in AML is associated with a good prognosis [24]. In the index study, in patients who were detected to have aberrant expression of CD19, most had an AML-ETO, which is a good risk feature in AML. The median duration from the diagnosis to treatment initiation was 4.5 days. The time from diagnosis to treatment beginning predicts survival in younger patients, and survival remains poor [25]. The time from the diagnosis to treatment initiation was lesser in this study compared to other studies reported from LMIC [26].

Our study has focused on the relevance of karyotyping in dissecting the role of chromosomal abnormalities in the risk stratification and prognosis of pediatric AML. Additionally, a few unbalanced cytogenetic abnormalities were observed in our study. Monosomy/deletion 7 has been reported to have a poor overall survival [27] and is frequent in the childhood myelodysplastic syndromes (MDS) [28]. Five patients (13%) had this particular abnormality. Trisomy 8 was not common (3%) in our findings. Trisomy 8 has been reported as single or is associated with any other abnormality [29]. Trisomy 8, as a sole abnormality, had a poor outcome in a recent BFM trial [1]. Monosomal and complex karyotypes have poor prognoses [1, 3, 30]. In this study, monosomal and complex karyotypes were uncommon, with occurrence in 5% and 3% of patients, respectively. Other uncommon unbalanced abnormalities observed were add51, add21; t(1;11); t(9;12); t(11;19). Tyagi et al reported a cytogenetic profile in 472 children with AML, and the most common cytogenetic abnormality observed was the loss of the Y chromosome in 12.9% of patients. In this cohort, trisomy 8 was the most frequent gain [31].

Subsequently, we targeted a few common mutations contributing to the pathogenesis of pediatric AML. The literature exhibiting a correlation of karyotype and molecular mutation with the survival in a pediatric population from a developing country is a deficit. The prime importance of inculcating advanced techniques in diagnosis and risk stratification can't be disregarded for therapeutic improvisations. AML-ETO and CBFB-MYH11 fusion is a core-binding factor (CBF) in the leukemias [32]. These abnormalities lead to the dysfunctionality of the CBF genes, which are involved in hematopoiesis and were observed in most cases (44%) in the index study. FLT3 is a tyrosine kinase that plays a ubiquitous role in the early stages of myeloid and lymphoid lineage development through different signaling pathways, including PI3K, RAS, and STAT5. Mutations in these receptors activate FLT3 kinase activity leading to AML [33]. FLT3 mutations were detected in 11% of patients, wherein FLT3-ITD has a poor prognosis and is more common, whereas FLT3-TKD, categorized under intermediate-risk, has less probability of occurrence [34]. NPM1 and CEBPA mutation generally occurs in patients with normal karyotypes and has a good prognosis in the disease [35, 36]. Our study observed NPM1 in 17.4% of CN-AML and 2.4% of CA-AML groups by fragment analysis.

In this cohort, the aberrant expression of CD19 was associated with the presence of cytogenetic abnormalities. In patients with an aberrant expression of CD19, all of those had t(8;21), and the majority were M2 subtype, as reported in other studies also [1]. CD7, CD11b, and CD36a were significantly present in the absence of molecular markers. It suggests that patients with negative molecular markers may have an expression of CD7, CD11b, and CD36a. The aberrant expression of CD19 was associated with the presence of molecular markers (AML-ETO).

The median event-free survival (EFS) in all patients (n=70, CN-AML, and CA-AML) was 11.9 months. In this study, in a follow-up of 40 months, 37% of patients died, out of which 81% died during induction. Treatment-related complications remain a significant cause of morbidity and mortality in childhood AML, even in developed countries [37, 38]. In the index study, sepsis was the main cause of death in 69% of cases. The induction mortality of our cohort is higher compared to data from high-income countries, in which it has been reported as less than 11% [39-41]. Infection-related mortality is expected to be higher in LMICs due to the lack of supportive care.

The forty months overall survival probability (pOS) was 58% in all patients, and there was no significant difference between CN-AML (pOS, 53%) vs the CA-AML groups (pOS, 63%). The index study reports a higher survival of AML patients, as reported in many studies from different institutes in India [42] (**Table 3**). From

**Table 3.** Comparative results of various studies of pediatric AML

| Studies | Study recruitment | Sample size | Age (years) | CR rates | EFS | OS |
|---|---|---|---|---|---|---|
| HICs | | | | | | |
| UK MRC-12 [43] | 1988-2002 | 758 | ≤16 | 92% | 5-y EFS: 56% | 5-y OS: 66% |
| AML-BFM 98 [44] | 1998-2003 | 473 | <18 | 88% | 5-y EFS: 49% | 5-y OS: 62% |
| AML99 [45] | 2000-2002 | 240 | ≤18 | 95% | 5-y EFS: 62% | 5-y OS: 76% |
| St Jude AML02 [41] | 2002-2008 | 216 | ≤21 | 94% | 3-y EFS: 63% | 3-y OS: 71% |
| LMICs | | | | | | |
| Kiem Hao et al [46] | 2010-2019 | 98 | ≤15 | 82.6% | 7-y EFS: 31.2 ± 3.7% | 7-y OS: 33.1 ± 4.1% |
| Yadav et al [47] | 2005-2010 | 51 | <18 | | | OS: 26% |
| Radhakrishnan et al [13] | 2008-2013 | 65 | <18 | 72% | 53-m: 28% | 53 m: 36% |
| Seth et al [48] | 2011-2015 | 71 | ≤18 | | 3-y EFS: 43% | 3-y OS: 54.7% |
| Index study | 2018-2020 | 70 | <18 | 100% | Median EFS: 11.9 m | 40-m pOS: 58% |

AML: Acute myeloid leukemia; BFM: Berlin-Frankfurt-Munster; CR: Complete remission; HICs: High-income countries; LMICs: Low-middle-income-countries; EFS: Event-free survival; OS: Overall survival.

high-income countries (HICs) (n=216-758) the reported CR rates is of 88-95%, and 5-year EFS and OS are 49-62% and 62-76%, respectively [41, 43-45]. On the contrary, the reported EFS and OS from LMICs (n=51-98) are 28-43% and 26-58%, respectively [13, 46-48]. These data suggest that survival outcomes in LMICs are lower to HICs. Pediatric patients with AML living in LMICs have not benefited from the advances as in high-income countries (HICs) [49]. Survival differences between LICs and HICs children have been attributed to late presentation to healthcare facilities, higher rates of relapse, abandonment of treatment, and higher rates of death from toxicity [50]. In our study, the median duration from onset of the symptoms to presentation to the hospital was 25 days, induction deaths were also high, and treatment was abandoned in 4% of cases.

Although pediatric AML is a low-risk genetic group, relapse remains common, up to 35%. Children at the highest risk of relapse related to poor genetic features have dismal outcomes and continue to require HSCT to achieve a cure, with one-third surviving at three years [51]. In the index study, one-third of patients had a relapse in a follow-up period of 40 months. The strengths of our study are its prospective recruitment of patients and comparison between CA-AML and CN-AML groups. The limitation of this study was the small sample size (n=70) and the short follow-up (40 months).

*Authors' view*

AML is a heterogenous hematologic malignancy with varied clinical presentations and dismal outcomes. Cyto-molecular testing is important in diagnosing, treating, and predicting the prognosis of AML. Supportive care (nutrition, judicious use of blood products, and infection control measures) and appropriate follow-up are critical in managing AML.

In conclusion, this cohort had a high prevalence of malnutrition, delayed presentation, and a higher prevalence of the FAB M2 subtype of AML than reported in the international literature. The majority of patients had abnormal karyotype/cytogenetics. FAB M2 subtype showed frequent aberrant expression of CD19. The absence of molecular markers may suggest the presence of CD7, CD11b, and CD36a expression. Induction mortality was higher than HICs, and sepsis was the leading cause of death. Although OS was lower than that of developed countries, however, the OS has improved compared to other centers in LMICs. Increased access to cyto-molecular tests and proper supportive care could enhance the survival of children with AML treated in LMICs.

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## Disclosure of conflict of interest

None.

## Abbreviations

ALL, Acute lymphoblastic leukemia; AML, Acute myeloid leukemia; CCG, Children Cancer Group; CA-AML, AML with abnormal/recurrent cytogenetic abnormalities; CN-AML, AML with normal cytogenetics; CD, Cluster of differentiation; CR, Complete remission; EMD, Extramedullary disease; EFS, Event free survival; FAB, French-American-British; HSCT, Hematopoietic bone marrow transplantation; IQR, Inter quartile range; LAR, Legally authorized representative; LMIC, Low-middle-income country; OS, Overall survival; PCR, Polymerase chain reaction; RT-PCR, Reverse transcription-polymerase chain reaction; TLC, Total leukocyte counts.

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## References

[1] Quessada J, Cuccuini W, Saultier P, Loosveld M, Harrison CJ and Lafage-Pochitaloff M. Cytogenetics of pediatric acute myeloid leukemia: a review of the current knowledge. Genes (Basel) 2021; 12: 924.

[2] Conneely SE and Stevens AM. Acute myeloid leukemia in children: emerging paradigms in genetics and new approaches to therapy. Curr Oncol Rep 2021; 23: 16.

[3] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B and Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447.

[4] Manola KN. Cytogenetics of pediatric acute myeloid leukemia. Eur J Haematol 2009; 83: 391-405.

[5] Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, De Bont ES, De Moerloose B, Dworzak M, Gibson BE, Hasle H, Leverger G, Locatelli F, Ragu C, Ribeiro RC, Rizzari C, Rubnitz JE, Smith OP, Sung L, Tomizawa D, van den Heuvel-Eibrink MM, Creutzig U and Kaspers GJ. Collaborative efforts driving progress in pediatric acute myeloid leukemia. J Clin Oncol 2015; 33: 2949-2962.

[6] Creutzig U, van den Heuvel-Eibrink MM, Gibson B, Dworzak MN, Adachi S, de Bont E, Harbott J, Hasle H, Johnston D, Kinoshita A, Lehrnbecher T, Leverger G, Mejstrikova E, Meshinchi S, Pession A, Raimondi SC, Sung L, Stary J, Zwaan CM, Kaspers GJ and Reinhardt D; AML Committee of the International BFM Study Group. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. Blood 2012; 120: 3187-3205.

[7] Ganguly S, Kinsey S and Bakhshi S. Childhood cancer in India. Cancer Epidemiol 2021; 71: 101679.

[8] van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, Dotti G, Griesinger F, Parreira A, Gameiro P, Diáz MG, Malec M, Langerak AW, San Miguel JF and Biondi A. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 concerted action: investigation of minimal residual disease in acute leukemia. Leukemia 1999; 13: 1901-1928.

[9] Huang Q, Chen W, Gaal KK, Slovak ML, Stein A and Weiss LM. A rapid, one step assay for simultaneous detection of FLT3/ITD and NPM1 mutations in AML with normal cytogenetics. Br J Haematol 2008; 142: 489-492.

[10] Elyamany G, Awad M, Fadalla K, Albalawi M, Al Shahrani M and Al Abdulaaly A. Frequency and prognostic relevance of FLT3 mutations in Saudi acute myeloid leukemia patients. Adv Hematol 2014; 2014: 141360.

[11] Indian Academy of Pediatrics Growth Charts Committee, Khadilkar V, Yadav S, Agrawal KK, Tamboli S, Banerjee M, Cherian A, Goyal JP, Khadilkar A, Kumaravel V, Mohan V, Narayanappa D, Ray I and Yewale V. Revised IAP growth charts for height, weight and body mass index for 5- to 18-year-old Indian children. Indian Pediatr 2015; 52: 47-55.

[12] WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl 2006; 450: 76-85.

[13] Radhakrishnan V, Thampy C, Ganesan P, Rajendranath R, Ganesan TS, Rajalekshmy KR and Sagar TG. Acute myeloid leukemia in children: experience from tertiary cancer centre in India. Indian J Hematol Blood Transfus 2016; 32: 257-261.

[14] Hossain MJ and Xie L. Sex disparity in childhood and young adult acute myeloid leukemia (AML) survival: evidence from US population data. Cancer Epidemiol 2015; 39: 892-900.

[15] Sung L, Lange BJ, Gerbing RB, Alonzo TA and Feusner J. Microbiologically documented infections and infection-related mortality in children with acute myeloid leukemia. Blood 2007; 110: 3532-3539.
[16] Laningham FH, Kun LE, Reddick WE, Ogg RJ, Morris EB and Pui CH. Childhood central nervous system leukemia: historical perspectives, current therapy, and acute neurological sequelae. Neuroradiology 2007; 49: 873-888.
[17] Støve HK, Sandahl JD, Abrahamsson J, Asdahl PH, Forestier E, Ha SY, Jahnukainen K, Jónsson ÓG, Lausen B, Palle J, Zeller B and Hasle H. Extramedullary leukemia in children with acute myeloid leukemia: a population-based cohort study from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). Pediatr Blood Cancer 2017; 64.
[18] Bakst RL, Tallman MS, Douer D and Yahalom J. How I treat extramedullary acute myeloid leukemia. Blood 2011; 118: 3785-3793.
[19] Paydas S, Zorludemir S and Ergin M. Granulocytic sarcoma: 32 cases and review of the literature. Leuk Lymphoma 2006; 47: 2527-2541.
[20] Lan TY, Lin DT, Tien HF, Yang RS, Chen CY and Wu K. Prognostic factors of treatment outcomes in patients with granulocytic sarcoma. Acta Haematol 2009; 122: 238-246.
[21] Pramanik R, Tyagi A, Chopra A, Kumar A, Vishnubhatla S and Bakhshi S. Myeloid sarcoma predicts superior outcome in pediatric AML; can cytogenetics solve the puzzle? Clin Lymphoma Myeloma Leuk 2018; 18: e249-e254.
[22] Zeller B, Glosli H, Forestier E, Ha SY, Jahnukainen K, Jónsson ÓG, Lausen B, Palle J, Hasle H and Abrahamsson J; NOPHO AML working group. Hyperleucocytosis in paediatric acute myeloid leukaemia-the challenge of white blood cell counts above 200 × $10^9$/l. The NOPHO experience 1984-2014. Br J Haematol 2017; 178: 448-456.
[23] Harani MS, Adil SN, Shaikh MU, Kakepoto GN and Khurshid M. Frequency of fab subtypes in acute myeloid leukemia patients at Aga Khan University Hospital Karachi. J Ayub Med Coll Abbottabad 2005; 17: 26-29.
[24] Pinheiro LHS, Trindade LD, Costa FO, Silva NL, Sandes AF, Nunes MAP, Correa CB, Almeida CAC, da Cruz GS, de Lyra Junior DP and Schimieguel DM. Aberrant phenotypes in acute myeloid leukemia and its relationship with prognosis and survival: a systematic review and meta-analysis. Int J Hematol Oncol Stem Cell Res 2020; 14: 274-288.
[25] Sekeres MA, Elson P, Kalaycio ME, Advani AS, Copelan EA, Faderl S, Kantarjian HM and Estey E. Time from diagnosis to treatment initiation predicts survival in younger, but not older, acute myeloid leukemia patients. Blood 2009; 113: 28-36.
[26] van Weelderen RE, Njuguna F, Klein K, Mostert S, Langat S, Vik TA, Olbara G, Kipng'etich M and Kaspers GJL. Outcomes of pediatric acute myeloid leukemia treatment in Western Kenya. Cancer Rep (Hoboken) 2022; 5: e1576.
[27] Trobaugh-Lotrario AD, Kletzel M, Quinones RR, McGavran L, Proytcheva MA, Hunger SP, Malcolm J, Schissel D, Hild E and Giller RH. Monosomy 7 associated with pediatric acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS): successful management by allogeneic hematopoietic stem cell transplant (HSCT). Bone Marrow Transplant 2005; 35: 143-149.
[28] Hasle H, Aricò M, Basso G, Biondi A, Cantù Rajnoldi A, Creutzig U, Fenu S, Fonatsch C, Haas OA, Harbott J, Kardos G, Kerndrup G, Mann G, Niemeyer CM, Ptoszkova H, Ritter J, Slater R, Starý J, Stollmann-Gibbels B, Testi AM, van Wering ER and Zimmermann M. Myelodysplastic syndrome, juvenile myelomonocytic leukemia, and acute myeloid leukemia associated with complete or partial monosomy 7. European Working Group on MDS in Childhood (EWOG-MDS). Leukemia 1999; 13: 376-385.
[29] Laursen AC, Sandahl JD, Kjeldsen E, Abrahamsson J, Asdahl P, Ha SY, Heldrup J, Jahnukainen K, Jónsson ÓG, Lausen B, Palle J, Zeller B, Forestier E and Hasle H. Trisomy 8 in pediatric acute myeloid leukemia: a NOPHO-AML study. Genes Chromosomes Cancer 2016; 55: 719-726.
[30] Chen X, Wang X, Dou H, Yang Z, Bi J, Huang Y, Lu L, Yu J and Bao L. Cytogenetic and mutational analysis and outcome assessment of a cohort of 284 children with de novo acute myeloid leukemia reveal complex karyotype as an adverse risk factor for inferior survival. Mol Cytogenet 2021; 14: 27.
[31] Tyagi A, Pramanik R, Chaudhary S, Chopra A and Bakhshi S. Cytogenetic profiles of 472 Indian children with acute myeloid leukemia. Indian Pediatr 2018; 55: 469-473.
[32] Sangle NA and Perkins SL. Core-binding factor acute myeloid leukemia. Arch Pathol Lab Med 2011; 135: 1504-1509.
[33] Grafone T, Palmisano M, Nicci C and Storti S. An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia: biology and treatment. Oncol Rev 2012; 6: e8.
[34] Daver N, Schlenk RF, Russell NH and Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia 2019; 33: 299-312.
[35] Ho PA, Alonzo TA, Gerbing RB, Pollard J, Stirewalt DL, Hurwitz C, Heerema NA, Hirsch B, Raimondi SC, Lange B, Franklin JL, Radich JP and Meshinchi S. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the

Children's Oncology Group. Blood 2009; 113: 6558-6566.
[36] Hollink IH, Zwaan CM, Zimmermann M, Arentsen-Peters TC, Pieters R, Cloos J, Kaspers GJ, de Graaf SS, Harbott J, Creutzig U, Reinhardt D, van den Heuvel-Eibrink MM and Thiede C. Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. Leukemia 2009; 23: 262-270.
[37] Creutzig U, Ritter J, Budde M, Sutor A and Schellong G. Early deaths due to hemorrhage and leukostasis in childhood acute myelogenous leukemia. Associations with hyperleukocytosis and acute monocytic leukemia. Cancer 1987; 60: 3071-3079.
[38] Molgaard-Hansen L, Möttönen M, Glosli H, Jónmundsson GK, Abrahamsson J and Hasle H; Nordic Society of Paediatric Haematology and Oncology (NOPHO). Early and treatment-related deaths in childhood acute myeloid leukaemia in the Nordic countries: 1984-2003. Br J Haematol 2010; 151: 447-459.
[39] Gupta S, Bonilla M, Valverde P, Fu L, Howard SC, Ribeiro RC and Sung L. Treatment-related mortality in children with acute myeloid leukaemia in Central America: incidence, timing and predictors. Eur J Cancer 2012; 48: 1363-1369.
[40] Creutzig U, Zimmermann M, Reinhardt D, Dworzak M, Stary J and Lehrnbecher T. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. J Clin Oncol 2004; 22: 4384-4393.
[41] Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J, Pounds S, Razzouk BI, Lacayo NJ, Cao X, Meshinchi S, Degar B, Airewele G, Raimondi SC, Onciu M, Coustan-Smith E, Downing JR, Leung W, Pui CH and Campana D. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. Lancet Oncol 2010; 11: 543-552.
[42] Arora RS and Arora B. Acute leukemia in children: a review of the current Indian data. South Asian J Cancer 2016; 5: 155-160.
[43] Gibson BE, Wheatley K, Hann IM, Stevens RF, Webb D, Hills RK, De Graaf SS and Harrison CJ. Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. Leukemia 2005; 19: 2130-2138.
[44] Creutzig U, Zimmermann M, Lehrnbecher T, Graf N, Hermann J, Niemeyer CM, Reiter A, Ritter J, Dworzak M, Stary J and Reinhardt D. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol 2006; 24: 4499-4506.
[45] Tsukimoto I, Tawa A, Horibe K, Tabuchi K, Kigasawa H, Tsuchida M, Yabe H, Nakayama H, Kudo K, Kobayashi R, Hamamoto K, Imaizumi M, Morimoto A, Tsuchiya S and Hanada R. Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: the AML99 trial from the Japanese Childhood AML Cooperative Study Group. J Clin Oncol 2009; 27: 4007-4013.
[46] Kiem Hao T, Van Ha C, Huu Son N and Nhu Hiep P. Long-term outcome of childhood acute myeloid leukemia: a 10-year retrospective cohort study. Pediatr Rep 2020; 12: 8486.
[47] Yadav SP, Ramzan M, Lall M and Sachdeva A. Pediatric acute myeloid leukemia: final frontier for pediatric oncologists in developing world. Pediatr Hematol Oncol 2011; 28: 647-648.
[48] Seth R, Pathak N, Singh A, Chopra A, Kumar R and Kalaivani M. Pediatric acute myeloid leukemia: improved survival rates in India. Indian J Pediatr 2017; 84: 166-167.
[49] Eden T, Pui CH, Schrappe M, Tognoni G and Masera G; Associazione Italiana di Ematologia ed Oncologia Pediatrica; Berlin-Frankfurt-Münster Study Group; Children's Oncology Group; German Cooperative Acute Lymphoblastic Leukaemia Study Group; Czech Pediatric Hematology; Dana-Farber Cancer Institute; Dutch Childhood Oncology Group; European Organization for Research and Treatment of Cancer; French Acute Lymphoblastic Leukemia Study Group; Japan Association of Childhood Leukaemia Study Group; Medical Research Council; United Kingdom Acute Lymphoblastic Leukaemia; Nordic Society of Pediatric Haematology and Oncology; St Jude Children's Research Hospital; Taiwan Pediatric Oncology Group; Tokyo Children's Cancer Study Group; International Society of Paediatric Oncology. All children have a right to full access to treatment for cancer. Lancet 2004; 364: 1121-1122.
[50] Howard SC and Wilimas JA. Delays in diagnosis and treatment of childhood cancer: where in the world are they important? Pediatr Blood Cancer 2005; 44: 303-304.
[51] Aplenc R, Meshinchi S, Sung L, Alonzo T, Choi J, Fisher B, Gerbing R, Hirsch B, Horton T, Kahwash S, Levine J, Loken M, Brodersen L, Pollard J, Raimondi S, Kolb EA and Gamis A. Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children's Oncology Group. Haematologica 2020; 105: 1879-1886.

**Supplementary Table 1.** Details of cyto-molecular analysis in pediatric AML with aberrant expression of CD19

| Patient ID | Age (years) | Gender | AML subtype | Karyotype | Cytogenetics | Molecular pattern |
|---|---|---|---|---|---|---|
| 1. | 7 | M | M4 | 46XY | t(8;21) del(9) | AML-ETO |
| 2. | 5 | M | M4 | 46XY | t(8;21) | AML-ETO, FLT3-ITD |
| 3. | 4 | M | M4 | Culture failure | Culture failure | FLT3-ITD |
| 4. | 12 | M | M2 | 45XY | t(8;21) | AML-ETO |
| 5. | 11 | F | M2 | 46XX | t(8;21) | AML-ETO |
| 6. | 7 | F | M2 | 46XX | t(8;21) | AML-ETO |
| 7. | 10 | M | M2 | Culture failure | Culture failure | AML-ETO |
| 8. | 3 | F | M2 | 46XX | t(8;21), t(2;21), der(21) | AML-ETO |
| 9. | 6 | F | M2 | 45X0 | t(8;21) | AML-ETO |
| 10. | 12 | M | M2 | 45X0 | t(8;21) | AML-ETO |
| 11. | 6 | F | M2 | 46XX | t(8;21) | AML-ETO |