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Cytogenetic characteristics of and prognosis for acute myeloid leukemia in 107 children

Wanzi Chen¹, Jinghui Yang², Ping Chen^{3,*}

Abstract

Background: Patients diagnosed with acute myeloid leukemia (AML) in childhood have a poor prognosis. A better understanding of prognostic factors will assist patients and clinicians in making difficult treatment decisions.

Objectives: To understand further the cytogenetic characteristics of and reassess the prognostic value of cytogenetic abnormalities in childhood AML.

Methods: Conventional karyotypes of 107 children with AML were analyzed retrospectively. The cases were divided into 4 groups based on genetic rearrangements; namely patients with: t(15;17)/*PML-RARA*; t(8;21)/*RUNX1-RUNX1T1* or inv(16)(p13;q22) and t(16;16)/*CBFB-MYH11*; -7 or complex karyotypes; normal karyotypes or other cytogenetic changes. Differences in age, sex, leukocyte count, event-free survival (EFS), and overall survival (OS) were analyzed.

Results: All French-American-British (FAB) subtypes of AML were detected in 107 patients. We successfully cultured 81 of 107 bone marrow specimens, of which 60 cases had abnormal karyotypes. The most common abnormal karyotypes were t(8;21) (17/81 cases), followed by t(15;17) (13/81 cases), -X/Y (10/81 cases). There were no significant differences ($P > 0.05$) in age, sex, or leukocyte counts between the 4 groups. The differences in 3-year EFS and OS between each pair were significant, except for groups of patients with t(8;21)/*RUNX1-RUNX1T1* and patients with normal karyotypes or other cytogenetic changes ($P = 0.054$).

Conclusions: Chromosomal abnormalities may provide important prognostic factors for AML in children and be helpful for risk stratification and individual treatment.

Keywords: childhood leukemias; cytogenetic analysis; karyotype; leukemia, myeloid, acute; prognosis

Acute myeloid leukemia (AML) is a group of highly heterogeneous hematological malignancies characterized by the uncontrolled proliferation of hematopoietic precursors and the loss of an ability to differentiate because of various genetic alterations. In recent years, the efficacy of treatment of AML in childhood has improved greatly [1, 2], and

survival rates have increased to 60%–75% [3, 4]. This is related to new diagnostic techniques, the improvements in therapy during induction and remission phases, favorable supportive care measures, and monitoring of minimal residual disease [3, 5, 6]. However, treatment-related mortality and relapse rates remain high [7].

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The complete biological characterization of childhood AML is an important goal. Currently, cytogenetics is considered to be one of the most important clinical prognostic factors [8]. The World Health Organization (WHO) classification for AML has become very important. According to the WHO classification, clinical criteria, cytogenetic and molecular abnormalities are incorporated and associated with the morphological and immunophenotypic characteristics used in the classification of the French-American-British (FAB) cooperative group. However, the WHO classification is based mainly on the experience of adult patients with AML. Consensus on its relevance and applicability to pediatric patients is still necessary.

Childhood AML is frequently characterized by chromosomal instability. Structural and numerical chromosomal abnormalities can be found in nearly 70% of childhood AML [3]. The cytogenetic and molecular characterization of AML has provided an improved understanding of leukemogenesis and AML risk stratification. Distinct cytogenetic aberrations leading to the formation of fusion genes have been found in more than 40% of childhood AML. Although hundreds of fusion genes have been associated with leukemia, only a small portion of these fusion events have been consistently recurrent and clinically characterized to the extent where they might be used effectively in the diagnosis and treatment of leukemia [9]. The prevalence of characterized gene fusions with clinical implications varies among different hematological malignancies. Specific leukemic gene rearrangements require an accurate diagnosis to determine a proper treatment regimen. Conventional karyotyping has revealed the frequent occurrence of large chromosomal gains or losses and the analysis is time-consuming, thus preventing immediate patient management [10]. However, conventional quantitative real-time polymerase chain reaction (qPCR) or fluorescence in situ hybridization (FISH) employed to detect the fusion genes is more accurate, sensitive, and rapid. Gene abnormalities, particularly several chromosomal rearrangements have been associated with morphological subgroups and were recognized as important parameters for diagnostic and follow-up purposes with implications for defining risk groups for relapse and selecting patients who need more intensive treatment [11, 12]. In the present study, we aimed to understand further the cytogenetic characteristics of and reassess the prognostic value of the main cytogenetic abnormalities in childhood AML as this would be helpful for clinicians to optimize therapeutic schedules. We summarized and analyzed the clinical data and cytogenetic analysis results of 107 children diagnosed with AML in Fujian Medical University Union Hospital, Fuzhou, Fujian, China.

Methods

Patients and groups

This retrospective study was approved by the ethics review board of the Fujian Medical University Union Hospital (2019KY123) and conducted in accordance with the principles outlined in the contemporary revision of the Declaration of Helsinki of 1964 (World Medical Association) incorporating the most recent (2013) and earlier amendments. Requirements for informed consent were waived as the study was a retrospective analysis, and this would not increase any risk to the patients, or interfere with their diagnosis or treatment, and the privacy of the patients is protected.

We conducted retrospective analyses of 107 cases of childhood AML diagnosed between October 2012 and March 2017 at Fujian Medical University Union Hospital. Briefly, 67 cases were in male and 40 cases were in female children whose ages ranged from 9 to 168 months, with a median of 74 months. The diagnostic criteria, risk grouping criteria, and treatment plans were based on the AML-2010 advice issued by the Hematology Group in Pediatrics Branch of the Chinese Medical Association [13]. According to primary cytogenetic abnormalities, the patients were divided into 4 groups; namely those with: *t(15;17)/PML-RARA*; *t(8;21)/RUNX1-RUNX1T1* or *inv (16)(p13;q22)* and *t(16;16)/CBFB-MYH11*; -7 or complex karyotypes; normal karyotypes, or other cytogenetic changes.

Patient data

We curated routine cytogenetic data including the results of karyotype, real-time fluorescent quantitative (qPCR), and FISH detection of the leukemia fusion genes *PML-RARA*, *RUNX1-RUNX1T1*, and *CBFB-MYH11*. Karyotypes were analyzed by conventional culture methods and R banding. The chromosomes were prepared using direct and short-term culture methods with about $1-2 \times 10^6/\text{mL}$ cells of bone marrow anticoagulated with heparin, then identified and described in accordance with the International System for Human Cytogenetic Nomenclature (ISCN, 2016). FISH analysis was performed with dual-color, dual fusion FISH probes for *PML-RARA*, and *RUNX1-RUNX1T1*, break-apart probes for *CBFB* and *MLL* (Beijing Jin Bu Jia Biotechnology) according to the manufacturer's instructions. When gene fusion occurred, two yellow fusion signals are produced by the proximity or superposition of green and red chromosomes, one red and one green (2Y1R1G) signal are presented. If not, two red and two green signals are presented (2R2G). When gene breakage occurred, the one yellow chromosome breaks

apart as one red and one green (1R1G) signal, and one yellow signal is presented (1Y1R1G). If not, two yellow signals are presented (2Y). MLL does not have a fusion gene, so *PML-RARA*, *RUNX1-RUNX1T1*, and *CBFB-MYH11* leukemia fusion genes alone were detected by qPCR using a leukemia fusion gene detection kit (Shanghai Bio Bad Biotechnology) according to the manufacturer's instructions.

Treatment

We collected treatment information for all patients, which had been prescribed by clinicians according to the AML-2010 advice issued by the Hematology Group in Pediatrics Branch of the Chinese Medical Association [13]. Briefly, the treatment regimen in patients with t(15;17)/*PML-RARA* included induction therapy: total trans-retinoic acid (ATRA) + daunorubicin (DNR), followed by consolidated treatment with DA (DNR + Ara-C), homoharringtonine (HRT) + cytosine arabinoside (Ara-C), and arsenic trioxide (ATO) for one course each. After remission, maintenance treatment was administered routinely with ATO, ATRA, 6-mercaptopurine (6-MP), and methotrexate (MTX) for a total of 5 cycles. All patients, despite patients with t(15;17)/*PML-RARA*, received conventional AML treatment in the hospital, and the main chemotherapy regimens were conducted as follows: induction chemotherapy with idarubicin (IDA) + Ara-C. Subsequently, treatment after radical remission was as follows: (1) Administration of 3 treatment courses of medium-dose Ara-C + DNR at 3–4 week intervals. (2) HA scheme: 2 courses of HRT 3 + Ara-C. (3) EA scheme: 3 sequential sessions of Etoposide Vp-16 + Ara-C. After complete remission (CR), 10 children who were eligible for a transplant having a suitable donor, and were willing to receive the transplantation treatment received an allogeneic hematopoietic stem cell transplant, and the other children received unity of order combined chemotherapy.

Follow-up

To identify treatment outcomes, participants were followed over time using a variety of methods, including telephone interviews, medical examiner reports, and computer-based patient records. Follow-up was from 2012 to June 31, 2018, with a median follow-up time of 32 (1–70) months. The morphological CR, which mainly included a morphological leukemia-free state (bone marrow <5% blasts in an aspirate with spicules and no blasts with Auer rods or persistence of extramedullary disease), absolute neutrophil count $>1 \times 10^9$ /mL, and platelets 100×10^9 /mL and no residual evidence of extramedullary disease. The event-free survival (EFS) period

was defined as the time from diagnosis to the first incident (including recurrence or death during CR) or the time of the last follow-up. Overall survival (OS) was defined as the time to death by any cause.

Statistical analyses

Data were processed using PASW Statistics for Windows (version 18.0; SPSS) and R software (survival and survminer R). The counting data were expressed by the rate and the measurement data by the median number. Sample rates were compared using a χ^2 test. Univariate and multivariate COX regression analysis was performed. Survival was analyzed using a Kaplan–Meier method and log-rank tests. We followed the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement [14]. Differences were considered to be significant when $P < 0.05$.

Results

FAB cooperative group subtype classification

Among the 107 children, there were 9 cases of M0, 6 cases of M1, 26 cases of M2, 13 cases of M3, 2 cases of M4, 47 cases of M5, 3 cases of M6, and 1 case of M7. The percentage of M5 was the highest, up to 44%. The percentage of M2 was 24%, second to that of M5. The percentage of M3 was 12%. The distribution of each subtype is shown in **Figure 1**.

Genetic abnormalities in childhood AML

Cells in bone-marrow specimens from 81 cases (75%) in 107 children were cultured successfully. Specifically, 21 cases had normal karyotypes (26%) and 60 were abnormal (74%). Among the 60 abnormal cases, t(8;21) had the highest percentage (18/81 cases), followed by t(15;17) (13/81 cases) and –X/Y (10/81 cases). In the present study, all –X/Y occurred in t(8;21) cases. Besides, 3 cases with normal karyotypes and 8 cases with no split-phase were found to be positive for *RUNX1-RUNX1T1* fusion genes by FISH and PCR. Therefore, for t(8;21)/*RUNX1-RUNX1T1*, the percentage was actually 27% (29/107 cases), with 18 cases having concordant cytogenetic/FISH/PCR findings. Other chromosomal (structural or numerical) abnormalities affected chromosomes: 7 (7/81 cases), 4 (5/81), 9 (5/81), 11 (5/81), 3 (4/81), 5 (4/81), 2 (3/81), 6 (3/81), 12 (3/81), 13 (3/81), 16 (2/81), 20 (2/81), 22 (2/81), 1 (1/81), and 10 (1/81). Two FISH results are presented in

Figure 1. FAB cooperative group subtype classification of the 107 cases of AML in childhood. AML, acute myeloid leukemia; FAB, French-American-British.

Figure 2 and the correlation between AML cytogenetic abnormalities and FAB cooperative group subtype classification in children is shown in **Table 1**.

There were 13 children with *t*(15;17)/*PML-RARA*. *Inv*(16), *t*(16;16), or *t*(8;21) were reproducible genetic abnormalities involving core binding factors and had good prognoses. Patients with *t*(8;21)/*RUNX1-RUNX1T1* or *inv*(16)(p13;q22) and *t*(16;16)/*CBFB-MYH11* had 31 cases, 2 cases with *inv*(16)/*CBFB-MYH11*, and 29 cases with *t*(8;21)/*RUNX1-RUNX1T1*, of which 10 were accompanied by sex chromosome deletion. In patients with -7 or complex karyotypes, 25 cases had complex karyotypes or chromosome 7 abnormalities. Those with -7, 7q-, and complex karyotypes had poor prognoses. In patients with normal karyotypes or other cytogenetic changes, there were 16 cases with normal karyotypes and 7 cases of other karyotype abnormalities, including 2 cases of balanced translocation, *t*(6;17)(p24;q11) and *t*(11;12)(q13;q13). Abnormal cases of chromosome numbers were +8 (4 cases) and +3 (1 case), respectively.

Analysis of curative rate and prognostic factors

In the following survival analysis, we excluded 21 patients who gave up treatment after diagnosis. Three patients with negative FISH and PCR results died early, with karyotypes 47, XX,

+21[4]/48, idem, +8[16]; 46, XX[20]; and no split phase. Of the 73 patients receiving treatment, the CR rate of treatment was 70% in the first course, rising to 94% in the second course. After CR, 10 children underwent allogeneic hematopoietic stem cell transplantation, with one death due to transplantation-related complications. The other children were treated with combined chemotherapy. The morphological CR rate of the groups in the first course was compared, with significant differences between patients with *t*(15;17)/*PML-RARA* and those with *t*(8;21)/*RUNX1-RUNX1T1* or *inv*(16)(p13;q22) and *t*(16;16)/*CBFB-MYH11*, patients with normal karyotypes or other cytogenetic changes and patients with -7 or complex karyotypes ($P < 0.001$, $= 0.006$, and < 0.001 , respectively). Both patients with *t*(8;21)/*RUNX1-RUNX1T1* or *inv*(16)(p13;q22) and *t*(16;16)/*CBFB-MYH11* and patients with normal karyotypes or other cytogenetic changes showed moderate survival and were therefore combined. Kaplan-Meier survival analysis showed that the differences in 3-year OS and EFS were statistically significant ($\chi^2 = 17.16$, $P < 0.001$ and $\chi^2 = 12.51$, $P = 0.002$). The 3-year EFS and OS rates for patients with *t*(15;17)/*PML-RARA* were greater than 89%, significantly higher than those for patients with *t*(8;21)/*RUNX1-RUNX1T1* or *inv*(16)(p13;q22) and *t*(16;16)/*CBFB-MYH11* + patients with normal karyotypes or other cytogenetic changes and patients with -7 or complex karyotypes ($P = 0.015$, < 0.001 , and 0.027 , 0.001 , respectively). While the

Figure 2. FISH results. **A.** *RUNX1-RUNXIT1* fusion, 2Y1R1G, $\times 1000$, **B.** *MLL* breakage, 1Y1R1G, $\times 1000$. When gene fusion occurred, two yellow fusion signals are produced by the proximity or superposition of green and red chromosomes, one red and one green (2Y1R1G) signal are presented. If not, two red and two green signals were presented (2R2G). When gene breakage occurred, one yellow chromosome breaks apart as one red and one green (1R1G) signal, and one yellow signal is presented (1Y1R1G). If not, two yellow signals are presented (2Y). Scale bars indicate 10 μm . FISH, fluorescence in situ hybridization.

3-year EFS and OS rates of patients with -7 or complex karyotypes were 22% and 30% respectively. There was a significant difference between patients with $t(8;21)/RUNX1-RUNXIT1$ or $inv(16)(p13;q22)$ and $t(16;16)/CBFB-MYH11+$ patients with normal karyotypes or other cytogenetic changes and patients with -7 or complex karyotypes ($P = 0.004, 0.001$). The results are presented in **Figures 3** and **4**. There were no significant differences in 3-year EFS and OS rates between patients with

Table 1. Relationship between cytogenetic abnormalities and FAB subtypes.

Cytogenetic abnormalities	FAB subtype (case)	Detection of fusion gene	
		FISH	RT-qPCR
$t(15;17)$	APL (13)	+ (all)†	+ (all)†
$t(8;21)$	M2 (3), M5 (5)	+ (all)	+ (all)
$t(8;21), -X/Y$	M2 (7), M5 (3)	+ (all)	+ (all)
$inv(16)/t(16;16)$	M4 (2)	+ (all)‡	+ (all)‡
Complex karyotypes	M0 (2), M1 (2), M2 (7), M5 (11), M6 (2), M7 (1)	– (all)	– (all)
-7	M0 (1)	– (all)	– (all)
Normal karyotypes	M0 (2), M2 (2), M5 (10), M6 (1)	+ (3)§	+ (3)§
Others	M0 (3), M5 (4)	– (all)	– (all)
No split phase	M0 (1), M1 (4), M2 (7), M5 (14)	+ (8)§	+ (8)§

Complex karyotypes were defined as karyotypes involving 3 or more chromosomal abnormalities; others abnormality include 1 case of $t(6;17)$, 1 case of $t(11;12)$, 4 cases of $+8$, 1 case of $+3$. The leukemia fusion genes including *PML-RARA*, *RUNX1-RUNXIT1*, and *CBFB-MYH11* were detected by fluorescence in situ hybridization or quantitative real-time polymerase chain reaction.

– fusion gene negative, + fusion gene positive, †*PML-RARA* fusion gene positive, ‡*CBFB-MYH11* fusion gene positive, §*RUNX1-RUNXIT1* fusion gene positive.

FAB, French-American-British cooperative group; FISH, fluorescence in situ hybridization; RT-qPCR, quantitative real-time polymerase chain reaction.

$t(8;21)/RUNX1-RUNXIT1$ or $inv(16)(p13;q22)$ and $t(16;16)/CBFB-MYH11$ and patients with normal karyotypes or other cytogenetic changes ($P = 0.054, 0.055$). Despite the removal of 2 cases of $inv(16)$ from patients with $t(8;21)/RUNX1-RUNXIT1$ or $inv(16)(p13;q22)$ and $t(16;16)/CBFB-MYH11$, there was no significant difference between the patients with $t(8;21)/RUNX1-RUNXIT1$ or $inv(16)(p13;q22)$ and $t(16;16)/CBFB-MYH11$ before and after the removal. However, the morphological CR rate for the first course of treatment in patients with $t(8;21)/RUNX1-RUNXIT1$ or $inv(16)(p13;q22)$ and $t(16;16)/CBFB-MYH11$ was significantly higher than that for patients with normal karyotypes or other cytogenetic changes ($P = 0.014$).

According to our cytogenetic groupings, there were no statistically significant differences in age, sex, or leukocyte count at initial diagnosis (**Table 2**). Further, we evaluated the impact on survival and prognostic factors on cytogenetics, FAB types, age, sex, and white blood cell (WBC) counts, morphological CR for the first course, and bone-marrow transplant by Cox regression analysis. The results showed that FAB cooperative group subtypes, age, sex, morphological CR in the

Figure 3. The OS curves are shown for the 4 groups of childhood AML. Group A, AML with t(15;17)/*PML-RARA*; Group B, AML with t(8;21)/*RUNX1-RUNX1T1* or inv(16) and t(16;16)/*CBFB-MYH11*; Group C, AML with -7 or complex karyotypes; Group D, AML with normal or other cytogenetic changes. AML, acute myeloid leukemia; OS, overall survival.

first course, and bone marrow transplantation did not correlate with survival time. However, in the cytogenetics group ($P = 0.003$, <0.001 , 0.003) and WBC count $\geq 100 \times 10^9/L$ ($P = 0.040$) had significant correlations with survival time. The multivariate COX regression analysis is shown in **Table 3**. The hazard function ratio (HR) shown indicated that the risk of death for patients with -7 or complex karyotypes was 22.65-fold greater than for patients with t(15;17)/*PML-RARA*, and the risk of death for patients with WBC counts $\geq 100 \times 10^9/L$ was 2.06-fold greater than for WBC counts $<100 < 10^9/L$.

Relationships between OS for children with t(8;21)/*RUNX1-RUNX1T1* and FAB subtypes

In the present study, 18 cases presented with t(8;21); 11 cases lacking t(8;21) were positive for *RUNX1-RUNX1T1* fusion genes by FISH or PCR. Therefore, there were 29 total cases of t(8;21)/*RUNX1-RUNX1T1*, divided into 13 cases of FAB-M5 and 16 cases of FAB-M2. The OS for the 2 groups was significantly different ($P = 0.024$) (**Figure 5**).

Discussion

The ratio of male to female pediatric patients was 1.68:1 for the 107 cases analyzed in the study, similar to the ratio observed for adult AML. The most common FAB cooperative group subtype was M5, followed by M2. The incidence of *RUNX1-RUNX1T1* gene fusion in FAB-M5 in our present study was higher than that observed outside China. This might be the result of differences between different ethnic and regional groups for drug tolerances and biological characteristics of leukemic cells. The result of Cox regression analysis showed that FAB subtypes, age, sex, morphological CR in the first course, and bone marrow transplantation were not associated with survival time; however, cytogenetics and WBC count $\geq 100 \times 10^9/L$ were associated with survival time. According to our grouping based on cytogenetic manifestations, there were no significant differences in age, sex, and leukocyte count at the initial diagnosis between any group. Currently, cytogenetics is considered as an important factor for clinical prognosis. For patients with t(8;21), t(16;16)/

Figure 4. The EFS curves are shown for the 4 groups of childhood AML. Group A, AML with t(15;17)/*PML-RARA*; Group B, AML with t(8;21)/*RUNX1-RUNX1T1* or inv(16) and t(16;16)/*CBFB-MYH11*; Group C, AML with -7 or complex karyotypes; Group D, AML with normal or other cytogenetic changes. AML, acute myeloid leukemia; EFS, event-free survival.

inv(16), t(15;17), the prognosis was relatively better, while for complex karyotypes -5/del(5q), -7, 11q23, t(3;3)/inv(3), t(6;9), and t(9;22), the prognosis was poor [15, 16]. In the present study, the treatment after diagnosis was abandoned in 21 children, and so the treatment rate was 81%. The CR rate of treatment for the first course was 70%. The remission rates for patients with t(15;17)/*PML-RARA* and t(8;21)/*RUNX1-RUNX1T1* were significantly higher than those for patients with -7 or complex karyotypes and patients with normal karyotypes or other cytogenetic changes, which consistent with the report by Ruan et al. [17]. In the present study, the detection rate of abnormal karyotypes was 74%, slightly lower than that reported by Sandahl et al. [18]. The t(8;21) abnormal karyotype had the highest incidence, followed by t(15;17), -X/Y, as consistent with reports of other studies in China and elsewhere [19, 20]. The 11q23 rearrangement in children was quite common internationally [21], but the 11q23 rearrangement had been detected in only 5 cases in our study, with a detection rate of 6%. It might be associated with

the diversity of rearrangement for the large number of 11q23/*MLL* translocation partner genes. Some abnormal rearrangements are difficult to detect, and the sensitivity of R banding is limited. Therefore, FISH and RT-PCR should be combined to improve the detection rate of *MLL* gene abnormalities.

According to our grouping based on cytogenetic manifestations, the results showed no statistically significant difference in age, sex, and leukocyte count at the initial diagnosis. A comparison showed that the morphological CR rate in the first course of treatment, 3-year EFS and OS rates for patients with t(15;17)/*PML-RARA* were significantly higher than those for patients with t(8;21)/*RUNX1-RUNX1T1* or inv(16)(p13;q22) and t(16;16)/*CBFB-MYH11* + patients with normal karyotypes or other cytogenetic changes and patients with -7 or complex karyotypes. The reported cure rate of AML in children ranges from 70% to 90% [22]. In our study, the cure rate difference between patients with t(8;21)/*RUNX1-RUNX1T1* or inv(16)(p13;q22) and t(16;16)/*CBFB-MYH11* + patients with normal karyotypes or other cytogenetic changes and

Table 2. Clinical characteristics of 92 patients with AML cytogenetic abnormality

Group	Case	Male/female	<i>P</i>	Median age in months (range)	<i>P</i>	WBC ($\times 10^9/L$)	<i>P</i>	Morphological CR rate in first course (%)	<i>P</i>
A	13	7/6	0.54† 0.93‡ 0.45§	62.4 (36–168)	0.32† 0.84‡ 0.86§	10.1 $\pm$ 13.5	0.21† 0.33‡ 0.55§	92	0.046†* 0.017‡* 0.10§
B	31	16/15	0.53 0.10¶	78.0 (32–166)	0.15 0.18¶	43.0 $\pm$ 62.2	0.93 0.68¶	80	0.55 0.83¶
C	25	15/10	0.31††	71.0 (20–158)	0.99††	25.8 $\pm$ 28.8	0.92††	68	0.47††
D	23	17/6	/	66.0 (10–168)	/	35.3 $\pm$ 41.5	/	58	/

AML, acute myeloid leukemia; CR, cure rate; WBC, white blood cell count.

Group A, AML with t(15;17)/*PML-RARA*; Group B, AML with t(8;21)/*RUNX1-RUNX1T1* or inv(16) and t(16;16)/*CBFB-MYH11*; Group C, AML with –7 or complex karyotypes; Group D, AML with normal or other cytogenetic changes.

†Group A vs. Group B.

‡Group A vs. Group C.

§Group A vs. Group D.

||Group B vs. Group C.

¶Group B vs. Group D.

††Group C vs. Group D.

**P* < 0.05.

Table 3. Univariate and multivariate Cox regression analyses for overall survival

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Karyotype				
A	1.0		1.0	
B	6.52 (0.84–50.60)	0.07	7.27 (0.91–58.11)	0.06
C	20.26 (2.57–159.75)	0.004*	22.65 (2.73–187.84)	0.004*
D	7.44 (0.91–61.16)	0.06	5.72 (0.65–50.78)	0.12
Sex				
Male	1.0		1.0	
Female	0.88 (0.43–1.81)	0.73	0.26 (0.31–1.39)	0.26
Age (month)				
<12	1.0		1.0	
≥ 12	0.22 (0.05–0.93)	0.25	0.11 (0.02–0.60)	0.19
WBC ($\times 10^9/L$)				
<100	1.0		1.0	
≥ 100	1.87 (0.65–5.32)	0.04*	2.06 (0.69–6.08)	0.011*
Morphological CR in first course				
Not CR	1.0		1.0	
CR	0.89 (0.26–1.12)	0.10	0.69 (0.32–1.46)	0.33
Marrow transplantation				
Yes	1.0		1.0	
No	1.70 (0.41–7.13)	0.47	2.44 (0.56–10.63)	0.24

AML, acute myeloid leukemia; CI, confidence interval; CR, cure rate; HR, hazard ratio; WBC, white blood cell count.

Group A, AML with t(15;17)/*PML-RARA*; Group B, AML with t(8;21)/*RUNX1-RUNX1T1* or inv(16) and t(16;16)/*CBFB-MYH11*; Group C, AML with –7 or complex karyotypes; Group D, AML with normal or other cytogenetic changes.

**P* < 0.05.

Figure 5. Comparison of overall survival in FAB-M5/M2 with t(8;21)/*RUNX1-RUNX1T1*. FAB, French-American-British.

patients with -7 or complex karyotypes was significant, which further confirmed an improved prognosis of AML patients with t(15;17), t(8;21), and inv(16) [23, 24]. Chromosomal abnormalities, including complex karyotypes, $-7/\text{del}(7q)$, $-5/\text{del}(5q)$, t(9;11), and 11q23 gene rearrangement, suggest poor or extremely poor prognoses, which is consistent with other reports [25, 26].

In the present study, cells were cultured from bone marrow in 24 h short-term culture, and karyotypes were analyzed by R banding. Further, FISH and PCR were used to detect *RUNX1-RUNX1T1* fusion genes. Our results showed that 18 cases with t(8;21) were detected by karyotype analysis; however, 11 cases with cell-culture failure or without abnormal chromosome detection were detected by FISH and PCR, and they were still positive for *RUNX1-RUNX1T1*. The combined detection by FISH and PCR increased the detection rate of *RUNX1-RUNX1T1* by 12%. Of 26 patients with AML-M2, 11 cases with t(8;21) or *RUNX1-RUNX1T1* fusion genes were detected, accounting for 58%. This percentage is slightly lower than that reported by

He et al. [27]. The EFS for patients with FAB-M5 featuring t(8;21)/*RUNX1-RUNX1T1* was higher than that for patients with FAB-M2 with t(8;21)/*RUNX1-RUNX1T1*. The present study found that the proportion of FAB-M5 in children with AML was relatively high, even higher than the proportion of FAB-M2. Moreover, t(8;21)/*RUNX1-RUNX1T1* in FAB-M5 was 28% of cases. These proportions differ from those found by other studies in China and elsewhere. It was generally believed that *RUNX1-RUNX1T1* positivity correlates with a good prognosis, but with the continuous development and improvement of clinical testing technology, it was clinically found that the life cycle and CR for *RUNX1-RUNX1T1* positive patients with AML was not high. This might be related to the formation of *RUNX1-RUNX1T1* producing multiple proteins in the nucleus and cytoplasm [28, 29]. Some mutations, such as *FLT3* mutations [30] and miRNA overexpression [31], also affect the prognoses of *RUNX1-RUNX1T1* positive patients. There are about 25% of patients with t(8;21) or positive *RUNX1-RUNX1T1* fusion gene that also has c-*KIT* mutations, changing the prognosis from good

to moderate [32]. Mutations in *ASXL1* and *ASXL2* were detected in about 10% and 20% of patients with this type of AML, respectively, and the cumulative recurrence rate for these patients increased [33]. Therefore, prognosis should comprehensively account for morphological characteristics, clinical characteristics, biological characteristics, and gene analysis of AML.

Conclusion

There are multiple schemes for prognosis classification in China and internationally, and regional differences exist in the incidence of some abnormal karyotypes in AML. It is particularly important to clarify the relationship between chromosomal abnormalities and the prognosis of AML in children. The present study further confirmed that cytogenetic expression is an important prognostic factor for AML in childhood and a guide for individualizing AML treatment. Given the importance of cytogenetic detection in the diagnosis and treatment of leukemia, supplementary tests, such as FISH or RT-qPCR, or a combination of these, could be used in patients with undiagnosed leukemia (including patients with normal karyotype analysis and no split phase), which should improve the detection rate of cytogenetic abnormalities in providing more comprehensive, accurate, and reliable genetic information for clinicians.

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Data sharing statement: Statistical summaries of the data generated and analyzed for the present report are included in this published article. Further details of data that support the findings of the present study are available from the corresponding author on reasonable request after deidentification from any patient whose data are included in this article.

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