**TITLE PAGE**

**Article title**

Clinical features and prognosis of infant acute lymphoblastic leukemia in China: A single-center retrospective analysis

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**Word count**

Abstract word count：249

Text word count: 2587

**Number of tables and figures：**

Number of tables：2

Number of figures：3

**Short running title**

Clinical features and prognosis of infant acute lymphoblastic leukemia in China

**Keywords**

Acute lymphoblastic leukemia; Infant; chemotherapy; Hematopoietic stem cell transplantation; Prognosis

**Abbreviations key**

|  |  |
| --- | --- |
| abbreviation | full term or phrase |
| ALL | acute lymphoblastic leukemia |
| EFS | event-free survival |
| OS | overall survival |
| WBC | white blood cell count |
| CCLG | Chinese Children Leukemia Group |
| BFM | Berlin-Frankfurt-Munster |
| HSCT | hematopoietic stem cell transplantation |
| MRD | minimal residual disease |
| CNS | central nervous system |
| COG | Children's Oncology Group |
| CR | complete remission |

**Abstract**

**Background:** In this retrospective analysis, we investigate the clinical features and prognosis of 23 infant patients (< 1 year of age) diagnosed with acute lymphoblastic leukemia (ALL).

**Methods:** We used clinical data of 23 children diagnosed with infant ALL at the Department of Pediatric Hematology & Oncology, Wuhan Children’s Hospital, between 1st January 2014 and 30th September 2019. EFS and OS rate curves were computed using the Kaplan-Meier estimator. The impact of prognostic factors on outcome was analyzed using the Cox model.

**Results:** The median WBC was 46.14 (6.46–513) × 109/L at initial diagnosis. All 21 patients immunophenotyped by flow cytometry had B-lineage ALL. *KMT2A-*rearrangement was identified in 72.2% （13/18）patients. Mutation screening for 13 patients indicated 4 patients with *KRAS* mutations, 4 with *TTN* mutations, 2 with *NOTCH1* mutations, 2 with *PTPN11* mutations and 2 with *NRAS* mutations. Of 12 patients who received chemotherapy, complete remission was achieved for 83.3% patients after one course of remission induction. A total of 3 patients underwent related haploidentical allogeneic hematopoietic stem cell transplantation. The expected 2-year overall survival (OS) rate was 55.6 ± 15.2% and the expected event free survival rate (EFS) was 44.4 ± 15.7%. Univariate analysis revealed WBC > 100 × 109/L at initial diagnosis as a risk factor for poor OS and EFS.

**Conclusion:** Treatment of infant ALL with the standard childhood ALL regimen achieved an OS rate similar to patients with high-risk ALL, and WBC at initial diagnosis may be an important prognostic indicator.

**1 INTRODUCTION**

Infant acute lymphoblastic leukemia (ALL) refers to ALL occurring in children aged < 1 year at diagnosis. This accounts for less than 5% of childhood ALL 1. It was previously believed that infant ALL had low remission induction, high recurrence, rapid disease progression and poor treatment outcome. Infants aged < 1 year have demonstrated the lowest EFS and OS rates in previous childhood ALL studies. Clinical trials run by several international cooperative groups have shown that intensive chemotherapy according to different risk groups has marginally improved the survival rate of infant ALL 2,3. Despite these advances, prognosis of infant ALL remains significantly inferior to that of children aged > 1 year. The Chinese Children Leukemia Group (CCLG) ALL 2008 protocol, based on the Berlin-Frankfurt-Munster

(BFM) treatment backbone with some modifications, was designed to improve the outcome of childhood ALL. It was published in Recommendations for diagnosis and treatment of childhood acute lymphoblastic leukemia (4th edition) in China4. However, less previous reports have assessed its efficacy for infant ALL, and there remains a general lack of studies on the treatment and prognosis of patients with infant ALL in China. Therefore, we performed a retrospective analysis of the clinical data of 23 patients with infant ALL diagnosed at the Department of Pediatric Hematology & Oncology, Wuhan Children’s Hospital, 12 of whom were treated according to the ALL protocol(4th edition) in the past 5 years. The present study assesses the clinical features of infant ALL and their associations with prognosis, and provides a reference for establishing improved treatment regimens.

**2 METHODS**

**2.1 Patients**

This retrospective analysis used clinical data of 23 children diagnosed with infant ALL at the Department of Pediatric Hematology & Oncology, Wuhan Children’s Hospital, between 1st January 2014 and 30th September 2019. All patients were < 1 year of age and 12 received treatment. All children underwent a routine peripheral blood test, bone marrow cytological examination and flow cytometric immunophenotyping after admission to form a confirmed diagnosis. Further molecular and cytogenetic examinations were performed for some children. Diagnosis and treatment were based on the Recommendations for diagnosis and treatment of childhood acute lymphoblastic leukemia4. The 12 children who received treatment were divided into groups of moderate risk (*KMT2A*-germline) and high risk (*KMT2A*-rearrangement). Informed consent form was obtained from the guardian of each child before chemotherapy. The present study complied with the Declaration of Helsinki and was approved by the Review Board of Ethics Committee at Wuhan Children’s Hospital.

**2.2 Criteria for** **hematopoietic stem cell transplantation (HSCT)**

With reference to the Interfant-06 regimen 2, related haploidentical allogeneic HSCT was recommended for children who had appropriate donors after the first course of consolidation therapy, and met one of the following criteria: (1) white blood cell count (WBC) ≥ 300 × 109/L at initial diagnosis; (2) age < 6 months at initial diagnosis; (3) poor response to oral prednisone treatment or no remission of minimal residual disease (MRD) after induction therapy.

**2.3 Data collection**

The clinical data of the included children were collected from the hospital medical records, including age, sex, chief complaint, WBC at initial diagnosis, bone marrow cytology, flow cytometric immunophenotyping, molecular and cytogenetic characteristics, treatment regimens, complications during treatment and treatment outcome. Follow-up was performed via reexamination at the outpatient service or by telephone, and the date of last follow-up was March 31, 2020. All patient data was updated on March 31, 2020.

**2.4 Statistical analysis**

SPSS (version 26.0; IBM) was used to perform all statistical analyses. Event-free survival (EFS) and overall survival (OS) rate curves were computed using the Kaplan-Meier estimator and compared using the log-rank test. The impact of prognostic factors on outcome was analyzed using the Cox model and the Wald test. *P* < 0.05 was considered statistically significant.

**3 RESULTS**

**3.1 Patient Characteristics**

There were 12 boys and 11 girls (with a male/female ratio of 1.09:1) included in the present study. The median age was 5 months (24 days – 11 months), 2 (8.7%) were < 1 month of age, 10 (43.5%) were 1-6 months of age, and 11 (47.8%) were ≥ 6 months of age (Table 1).

The most common symptoms expressed as the chief complaint were pyrexia (9 patients, 39.1%), ochriasis (7 patients, 30.4%), and hemorrhagic spots on the skin (3 patients, 13.0%), while a small number of children visited the hospital due to abdominal distension, loss of appetite, crying, cough, nasal obstruction or a running nose. Hepatosplenomegaly was observed in 22 children (95.6%) and superficial lymphadenectasis was observed in 11 children (47.8%). One child had central nervous system (CNS) infiltration (grade II) at initial diagnosis.

The median WBC was 46.14 (6.46–513) × 109/L at initial diagnosis, with 4 patients (17.4%) presenting a WBC of ≥ 300 ×109/L, 3 (13.0%) of 100–300 × 109/L, and 16 (69.6%) < 100 × 109/L. The median hemoglobin was 73 (24–120) g/L, and the median platelet count was 56 (10–505) × 109/L.

Flow cytometric immunophenotyping performed for 21 children showed that all had B-lineage ALL. Bone marrow karyotyping performed for 17 children revealed abnormal results for 10 patients, and 6 had 11q23 abnormalities. Fusion gene detection was performed for 18 patients, among whom 7 (30.4%) were *KMT2A/AFF1*-positive, 3 (11.1%) were *KMT2A/MLLT10*-positive, 1 (5.5%) was *KMT2A/MLLT3*-positive, and 1 (5.5%) was *ETV6/RUNX1*-positive. Also, 2 children exhibited *KMT2A* rearrangement, detected by fluorescence *in situ* hybridization (FISH), but negative fusion gene detection. No abnormalities were detected for the remaining 4 children.

Mutations in leukemia-related genes were identified in 8/13 patients: *KRAS* mutations were identified in 4 patients, *TTN* mutations in 4 patients, *NOTCH1* mutations in 2 patients, *PTPN11* mutations in 2 patients, and *NRAS* mutations in 2 patients (table1).

**3.2 Outcome**

At the end of follow-up, 7/12 children who received treatment were alive, with an expected 2-year overall survival (OS) rate of 55.6 ± 15.2% and an expected 2-year event-free survival (EFS) rate of 44.4 ± 15.7%(Fig. 1). A total of 11 children did not receive further treatment; 5 of the 11 patients received oral prednisone or short-term chemotherapy, discontinued VDLD induction regimen, and died after 4-5 months; 5 did not receive any treatment and died within 1-2 months, and 1 was lost to follow-up. The remaining 12 children received chemotherapy based on the previously reported regimen 4, among whom 3 underwent related haploidentical allogeneic HSCT. Of the 12 children who received VDLD induction chemotherapy, 10 (83.3%) achieved complete remission, 1 (8.3%) achieved partial remission, and 1 (8.3%) achieved no remission.

Of the 9 children who did not receive HSCT, 3 experienced recurrence, with 1 case of bone marrow recurrence 10 months after diagnosis, 1 case of bone marrow recurrence 4 months after diagnosis, and 1 case of intracranial recurrence 1 year after diagnosis. A total of 6 patients were alive at the end of follow-up, with a median follow-up time of 25 (9-60) months. Among the 3 children who died, 1 died of recurrence 10 months after diagnosis, 1 died of recurrence 4 months after diagnosis, and 1 did not achieve remission after 4 courses of chemotherapy and 2 courses of CAR-T treatment and died 6 months after diagnosis.

Among the 3 children who received HSCT, 1 achieved disease-free survival after 30 months of follow-up, 1 died of severe hepatic sinusoidal obstruction syndrome and cytomegalovirus disease 2 months after transplantation, and 1 died of intracranial recurrence and intestinal rejection 2 months after transplantation.

By univariate analysis, WBC was identified as a risk factor for poor OS and EFS rate. Patients with a WBC ≥ 100 × 109/L had a worse OS (OR, 14.5; 95% CI, 1.47–143.36; *P* = 0.02) and EFS (OR, 14.5; 95% CI, 1.47–143.36; *P* = 0.02) than those with WBC < 100 × 109/L (Fig. 2 and 3). Meanwhile sex, age (< 6 months), severe anemia, abnormal platelet count, *KMT2A* gene rearrangement, adverse reaction to prednisone, and levels of MRD on days 15 and 33 had no significant influence on OS or EFS (*P* > 0.05).

4 **DISCUSSION**

Infant ALL is a rare type of hematologic malignancy. A total of 435 cases of ALL were diagnosed in the Department of Pediatric Hematology & Oncology, Wuhan Children’s Hospital between 1st January 1, 2014 and 30th September, 2019, only 23 (5.3%) of which were infant ALL. This is a similar proportion to that reported by1. The etiology of infant ALL remains unknown, but studies have shown that high birth weight (> 4 kg), and exposure to antitumor drugs, quinolones, alcohol, radiation or organic solvents during pregnancy may be high-risk factors for infant ALL 5,6. A case of t(4;11) B-linage infant ALL was reported in triplets in Turkey 7, and *KMT2A* rearrangement has been reported in identical twins 8, suggesting that the onset of infant ALL may occur *in utero*.

Around 80% of infant ALL cases present *KMT2A* rearrangement and are characterised by hyperleukocytosis at initial diagnosis. Hepatosplenomegaly, high risk of central nervous system (CNS) disease, CD10 negative early hematopoietic precursor phenotype, co-expression of myeloid and lymphoid antigen, and poor prognosis with a 4–5-year EFS of 37–49%, are usual for cases with *KMT2A* rearrangement. Infant ALL children without *KMT2A* rearrangement have a 4–5-year EFS of 69–95% 9. Until now, over 90 partner genes have been reported to fuse with *KMT2A*, among which *KMT2A/AFF1* (49%) is the most common rearrangement identified in infant ALL, followed by *KMT2A/MLLT1* (22%), *KMT2A/MLLT3* (16%), and *KMT2A/MLLT10* (6%). These types of rearrangement in KMT2A have no significant influence on infant ALL prognosis 10,11. In the present study, 13/18 (72.2%) children were found to have *KMT2A* rearrangements, including the 5 children who died after treatment. The 4 children without *KMT2A* abnormalities were all alive at the end of follow-up. In addition, 8 children were found to have mutations in leukemia-related genes, including *KRAS*, *NRAS*, and *PTPN11,* involved in the *PI3K/RAS* signaling pathway, which can promote the development of leukemia in a synergistic manner with *KMT2A* rearrangement. Children with these mutations are more likely experience recurrence 12.

Compared with the long-term EFS of ALL of > 80% 13,14, patients with infant ALL have a poor prognosis, with 4–6-year EFS and OS rates of 42–50% and 45–67%, respectively (Table 2). Multiple international infant ALL cooperative groups have suggested that the treatment regimens for infant ALL should be different from those for childhood ALL due to the unique biological characteristics of infant ALL 2,11,15-18. However, the IC-BFM 2002 trial for childhood ALL was used for the treatment of infant ALL and achieved a 5-year EFS of 58 ± 13% 19, which is supported by the 6-year EFS of the interfant-06 trial. In the present study, the Recommendations for diagnosis and treatment of childhood acute lymphoblastic leukemia (4th edition) was used, and a complete remission rate of 83.3% was achieved, as well as an expected 2-year OS rate of 55.6 ± 15.2%, and an EFS of 44.4 ± 15.7%, which are similar to the complete remission rate and OS and EFS rates with regimens used by the above cooperative groups. This suggests that the regimen for childhood ALL has a similar clinical effect to that of the infant ALL-specific regimen, achieving a similar OS rate to its use for children with high-risk ALL. Due to the small number of cases and the relatively short follow-up period, further studies with cases from multiple centers and longer observation periods are required.

In the Interfant-06 trial, *KMT2A* rearrangement, age < 6 months, WBC > 300 × 109/L at initial diagnosis, and poor prednisone response were demonstrated to be risk factors for prognosis 2. In the present study, univariate analysis of the 12 children who underwent treatment suggested that WBC > 100 × 109/L at diagnosis was a risk factor for poor OS (OR, 14.5; 95% CI, 1.47–143.36; *P* = 0.02) and EFS (OR, 14.5; 95% CI, 1.47–143.36; *P* = 0.02), while sex, age, hemoglobin, platelet count, prednisone response, and MRD levels on days 15 and 33 were not significantly associated with OS (*P* > 0.05) or EFS (*P* > 0.05). Surprisingly, *KMT2A* rearrangement was not identified as a risk factor for OS (*P* = 0.17) or EFS (*P* = 0.51), and further studies are required to determine whether this was due to the small number of cases or short follow-up time.

Patients with infant ALL usually experience recurrence within 1 year, with an extremely low remission rate after recurrence (~40%) and poor prognosis (3-year OS, 20%) 20. In the present study, 4 children who received treatment experienced recurrence (recurrence rate, 33%), which is similar to the recurrence rates reported in the Interfant-99/06 trials (34/37.5%) 2,11. Death due to recurrence in our study occurred in 3 cases in the present study, indicating the challenge that poses in the treatment of infant ALL. Two AML-like courses were added to the Interfant-06 trial at the stage of consolidation therapy: ADE (cytarabine, doxorubicin, and etoposide) and MAE (mitoxantrone, cytarabine, and etoposide). These were designed to increase the intensity of chemotherapy and prevent recurrence, however, EFS and OS rates were not improved due to the increase in infection-related deaths 2. Therefore, aiming to increase the intensity of chemotherapy may not be suitable for infant ALL. A UK study used blinatumomab prior to transplantation in 11 patients with infant ALL after first complete remission (CR1) or recurrence, achieving 3-year EFS and OS rates of 47% and 81%, respectively 21. The Children's Oncology Group (COG) and Interfant Group are also studying the safety and feasibility of azacitidine and blinatumomab treatment of infant ALL. Some novel treatment methods are under development, including FLT3 inhibitors, epigenetic agents, therapies targeting microenvironment interactions, immunotherapy, and therapies targeting the RAS pathway 22.

At present, HSCT is not recommended for *KMT2A*-germline patients with infant ALL after CR1 3. The JPLSG-MLL03 trial recommends HSCT for all children with *KMT2A* rearrangement and that HSCT is suitable (with CR and appropriate donors) within 4 months after CR1 to reduce the risk of early recurrence 16. Two parallel COG trials (CCG 1953; POG9407) showed similar 5-year survival rates of infants with KMT2A rearrangements who did or did not undergo transplantation 23. The Interfant-06 protocol recommends HSCT for infant ALL children with MRD ≥ 10−4 after consolidation therapy in the moderate risk group (with *KMT2A* rearrangement, > 6 months of age, WBC < 300 × 109/L at diagnosis, and good response to prednisone induction), resulting in a 4-year DFS of 19% with most children still experiencing recurrence 2. In the present study, 2 of 3 children who underwent related haploidentical allogeneic HSCT had *KMT2A* rearrangement and WBC > 300 × 109/L at initial diagnosis. Of these patients, 1 died of recurrence 2 months after transplantation and 1 died of transplantation-related cytomegalovirus disease 2 months after transplantation. The infant who survived had *KMT2A*-germline and underwent HSCT due to MRD > 10-3 after remission induction, and had not experienced recurrence after a 30-month follow-up. Further studies are required to determine the optimal timing and regimen of HSCT for infant ALL.

In conclusion, the OS and EFS rates for infant ALL in our center are 55.6 ± 15.2% and 44.4 ± 15.7%，respectively. Our data indicate that WBC > 100 × 109/L at initial diagnosis is a risk factor for poor OS and EFS. Treatment of infant ALL using infant ALL-specific regimens or standard childhood ALL regimens fail to achieve satisfactory clinical outcomes. Due to the rarity of this disease, further multicenter large-scale studies are required to improve the existing understanding of infant ALL, and the identification of novel targeted therapeutic drugs or immunotherapies is urgent.

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

Kaili Li collected and analysed the data, performed statistical analysis and wrote the manuscript; Ping Zhou collected the data; Yi Li performed statistical analysis and reviewed the paper; Jianxin Li, Hui Li, Fang Tao and Zhuo Wang recruited the patients and collected the data; Zhi Chen contributed to manuscript preparation; Hao Xiong designed the research and contributed to writing the manuscript.

**REFERENCES**

1. Tomizawa D. Infant ALL. Singapore: Kato, Motohiro; 2020.81-91

2. Pieters R, De Lorenzo P, Ancliffe P et al. Outcome of Infants Younger Than 1 Year With Acute Lymphoblastic Leukemia Treated With the Interfant-06 Protocol: Results From an International Phase III Randomized Study. *J Clin Oncol*. 2019;37(25):2246-2256

3. Kotecha RS, Gottardo NG, Kees UR, Cole CH. The evolution of clinical trials for infant acute lymphoblastic leukemia. *Blood Cancer J*. 2014;4:e200

4. Study Group of Hematology, Chinese Pediatric Society, Chinese Medical Association, Editorial Board of Chinese Journal of Pediat. Recommendations for diagnosis and treatment of childhood acute lymphoblastic leukemia (4th edition). *Chinese Journal of Pediatrics*. 2014;52(9):641-644

5. Felix CA, Lange BJ. Leukemia in infants. *Oncologist*. 1999;4(3):225-240

6. Zweidler-McKay PA, Hilden JM. The ABCs of infant leukemia. *Curr Probl Pediatr Adolesc Health Care*. 2008;38(3):78-94

7. Yaman-Bajin I, Aytac S, Kuskonmaz B et al. Infant lymphoblastic leukemia: a single centers 10 year experience. *Turk J Pediatr*. 2019;61(3):325-329

8. Ford AM, Ridge SA, Cabrera ME et al. In utero rearrangements in the trithorax-related oncogene in infant leukaemias. *Nature*. 1993;363(6427):358-360

9. Guest EM, Stam RW. Updates in the biology and therapy for infant acute lymphoblastic leukemia. *Curr Opin Pediatr*. 2017;29(1):20-26

10. Meyer C, Burmeister T, Groger D et al. The MLL recombinome of acute leukemias in 2017. *Leukemia*. 2018;32(2):273-284

11. Pieters R, Schrappe M, De Lorenzo P et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. *Lancet*. 2007;370(9583):240-250

12. Esposito MT. The Impact of PI3-kinase/RAS Pathway Cooperating Mutations in the Evolution of KMT2A-rearranged Leukemia. *Hemasphere*. 2019;3(3):e195

13. Bonaventure A, Harewood R, Stiller CA et al. Worldwide comparison of survival from childhood leukaemia for 1995-2009, by subtype, age, and sex (CONCORD-2): a population-based study of individual data for 89 828 children from 198 registries in 53 countries. *Lancet Haematol*. 2017;4(5):e202-e217

14. Cui L, Li ZG, Chai YH et al. Outcome of children with newly diagnosed acute lymphoblastic leukemia treated with CCLG-ALL 2008: The first nation-wide prospective multicenter study in China. *Am J Hematol*. 2018;93(7):913-920

15. Tomizawa D, Koh K, Sato T et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. *Leukemia*. 2007;21(11):2258-2263

16. Koh K, Tomizawa D, Moriya SA et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia. *Leukemia*. 2015;29(2):290-296

17. Hilden JM, Dinndorf PA, Meerbaum SO et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: report on CCG 1953 from the Children's Oncology Group. *Blood*. 2006;108(2):441-451

18. Salzer WL, Jones TL, Devidas M et al. Modifications to induction therapy decrease risk of early death in infants with acute lymphoblastic leukemia treated on Children's Oncology Group P9407. *Pediatr Blood Cancer*. 2012;59(5):834-839

19. Stary J, Zimmermann M, Campbell M et al. Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. *J Clin Oncol*. 2014;32(3):174-184

20. Driessen EM, de Lorenzo P, Campbell M et al. Outcome of relapsed infant acute lymphoblastic leukemia treated on the interfant-99 protocol. *Leukemia*. 2016;30(5):1184-1187

21. Clesham K, Rao VN, Bartram J et al. Blinatumomab for Infant Acute Lymphoblastic Leukaemia. *Blood*. 2020;

22. Brown P, Pieters R, Biondi A. How I treat infant leukemia. *Blood*. 2019;133(3):205-214

23. Dreyer ZE, Dinndorf PA, Camitta B et al. Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's Oncology Group. *J Clin Oncol*. 2011;29(2):214-222

**Figure legend**

**Figure 1.** Kaplan-Meier curve of the OS rate and EFS rate of 12 patients with infant ALL

**Figure 2.** The influence of WBC on the probability of OS of patients with infant ALL

**Figure 3.**  The influence of WBC on the probability of EFS of patients with infant ALL

**Table legend**

**Table 1.** Clinical and molecular features of patients with infant ALL

**Table 2.** Summary of results for infant ALL in recent clinical trials