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Objective: Acute mixed-lineage leukemia (AMLL) is characterized as the acute leukemia involved with acute myeloid cells and lymphoid cells at the same time. The AMLL is easily misdiagnosed because of a dual character involved with lymphoid and myeloid cells. At present, researches of AMLL in adults are more common. Only some are reported for children. Therefore, our aim was to study clinical characteristics of the childhood AMLL.

Findings: Fourteen cases of AMLL were screened from 185 cases of acute leukemia by morphology, immunology, cytogenetics and molecular (MICM). The rate of childhood AMLL accounted for 7.57% of pediatric acute leukemia (AL) diagnosed in the research period; white blood cell count in most of the patients was normal, the average value being $31.0 \times 10^9/L$ in the first visit. In the 14 cases of AMLL, 8 cases were B-Ly+/My+, 2 cases were T-Ly+/My+B, and 4 were T+B-Ly+/My+. Among them, nine cases received treatment. Consequently, 6 cases reached complete remission (CR); 1 case had not complete remission; 2 cases did not complete the treatment.

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Introduction

With the wide use of immunophenotyping technology in clinic, acute mixed-lineage leukemia (AMLL) has been gradually recognized and established as a rare type of leukemia in recent years [1]. It is characterized by existence of acute myeloid and lymphoid cells at the same time in the acute leukemia, and involved with lymphoid and myeloid cells as a dual character. Consensus criteria for the diagnosis of AMLL have been established in 1995. European Group for the Immunological Characterization of Leukemia (EGIL) has proposed a scoring system for the immunological classification of acute leukemia. According to characteristics of leukemia cells regarding antigen expression and cell source, AMLL phenotype can be further divided into biphenotypic, bilinear and biclonal types (including the conversion-type) [2]. Also, other researchers have advised morphological and cell chemistry characteristics to support ANLL of AMLL calling Ly+ANLL or My+ALL[1]. But in fact, the diagnosis of AMLL is very difficult, and its clinical significance and prognosis is not entirely clear.

Therefore, the clinical data of 14 cases of AMLL children admitted to our hospital during nearly 10 years were analyzed and the results are reported in the following.

Subjects and Methods

Subjects: From January 2000 to July 2009, 14 cases of AMLL children were selected by morphological and immunophenotyping methods from 185 cases of childhood acute leukemia (AL) admitted to the Department of Pediatrics, Tongji Hospital, Tongji Medical College of Science and Technology of Huazhong University. There were 12 males and 2 females; ages of patients were 1.5-12.0 (mean 7) years. Patients entered the study after their parents or guardians had signed informed consent. The study was approved by the Institutional Ethics Committee in our hospital.

Bone marrow morphology examinations: Bone marrow smears were analyzed by Wright's classification counts, and had staining exami-

nations, including inhibition test (NSE and NAF) for increase of non-specific esterase by peroxides (POX) and sodium fluoride, neutrophil alkaline phosphate staining (NAP) and glycogen staining (PAS) referenced to FAB criteria to determine the types.

Immunophenotyping: Bone marrow or peripheral blood (50uL) were detected by fluorescent isothiocyanate (FITC) / phycoerythrin (PE) / periteneum chlorophyll protein (Pre-CP) labeled antibodies, (all purchased from Becton Dickinson Company) which were added to the 20 μ L reaction tube, and 4°C response for 20-30 min; then added 2 ml erythrocyte lysine, at room temperature 10min after staining; PBS were washed 3 times, then added 0.5 ml PBS. All the samples were measured using FACScan model flow cytometer (BD USA Co) by the 488nm laser light. Each tube was revised to accommodate to 10000 cells via the standard calibration of fluorescent micro sphere by calibration instruments. *Cellquest* software was used for analysis. In virtue of CD45/SSC gating naive to discerning leukemia cells, the positive expression rate of hematopoietic cell antigens was analyzed to calculate the immature cells. The monoclonal antibodies of B lineage include CD10, CD19, CD20, CD22; T lineage include CD3, CD5, CD7; myeloid antigens include CD13, CD33, CD14, CD15; while stem cell antigen markers included CD34 and HLA-DR. The standard of leukemia cells diagnosis is positive antigen expression ($\geq 20\%$).

Chromosome analysis: Bone marrow specimens with heparin and phytohemagglutinin were cultured for 24 h. The R-banding was used by the heated method of (87.5°C) (Wright staining), and the karyotype was analyzed by the international human genetics nomenclature.

Fusion gene: 2-3 ml bone marrow with heparin was taken. The bone marrow fluid mononuclear cells were separated by ficoll liquid and fusion gene detected by reverse transcription-polymerase chain reaction (RT-PCR).

Findings

Clinical features: Fourteen cases of childhood AMLL from 185 AL cases accounted for 7.57%.

Twelve cases were males. Two (71.43%) female cases had clinical manifestation of fever, in 9 (64.29%) cases enlargement of superficial lymph nodes was found, 12 (85.71%) cases had hepatomegaly, 5 (35.71%) cases splenomegaly; 5 cases gum or skin bleeding. White blood cell count in 14 cases was $(166.5 \pm 1.34) \times 10^9/L$, in 8 cases normal, and in 2 cases more than $100 \times 10^9/L$ with a mean count $31.0 \times 10^9/L$. The average value of hemoglobin was 68.27 g/L (37-102 g/L) and the average platelet count was $80.14 \times 10^9/L$ (0-318) $\times 10^9/L$, while in 7 cases it was below $50 \times 10^9/L$.

FAB morphology typing of bone marrow cells: In 4 cases, 1 case was L1, and 3 cases L2; acute non-lymphoblastic leukemia (ANLL) were 6 cases, including 3 cases of acute myeloid leukemia (M2), 2 cases of acute monotypic leukemia (M5), and 1 case of acute myeloid-monastic leukemia (M4). Other 4 cases were diagnosed as AMLL on the morphology only. In 14 cases bone marrow consisted of 36.8% to 99.9% (average 73.73%) blast cells.

Immunophenotype: Immunophenotypic analysis was performed for all the 14 cases. This showed that 8 cases were of B-Ly+/My+ biphenotypic, 2 cases of T-Ly+/My+ biphenotypic, and 4 cases of T+B-Ly+/My+ biphenotypic type. 8 cases had double expression of myeloid and B lineage and 4 cases T+B-Ly+/My+ biphenotypic. In B antigens, 8 cases had CD19 positive expression, 3 cases CD10 positive, and 2 cases CD22 positive. The expression of myeloid and T lineage was in 2 cases and co-expression of T+B-Ly+/My+ in 4 cases. Positive expression of CD7 was seen in all the cases, and 4 cases of CD3 positive expression, 3 cases of CD5 expression. Myeloid antigen expression was positive in the 14 cases, in which there were 7 cases of CD13 positive expression, 6 cases of CD14 positive expression, 5 cases of CD33 positive expression, and 2 cases of CD15 positive expression. CD34 positive expression was seen in 2 cases and HLA-DR positive expression in 7 cases.

Chromosomes and fusion gene: Six cases were analyzed in AMLL chromosomes and fusion gene. 2 cases were found abnormal in number of chromosomes, 1 case of hyper diploid. 4 cases were found with structural abnormalities, 2 cases of chromosome translocation, 1 case of centric chromosome breakage and 1 case of mixed anomalies. Five cases were examined for fusion

gene, 3 cases had abnormal AMLex5/ETO, AML-ETO, MLL gene rearrangements.

Chemotherapy and sequelae: Because of no enough income of their parents, 5 cases did not receive chemotherapy, and 9 cases received chemotherapy as the only treatment. Five cases received VDLP (vincristine, daunorubicin, L-asparagine and dexamethasone) in the first course. 3 cases received DA (daunorubicin, Ara-C), DAE (daunorubicin, cytarabine, etoposide), and ARTA (all-trans retinoic acid) + DAE respectively. After the first course of chemotherapy, 6 cases achieved CR and 2 cases did not complete the chemotherapy, 1 case missing CR. In 6 cases of CR, 5 cases were B-Ly+/My+ biphenotypic leukemia, 1 case T-Ly+/My+ biphenotypic leukemia. Now (after 2-9 years follow up) 6 cases who achieved complete remission are alive. But 3 cases who didn't achieve complete remission died.

Discussion

AMLL is a rare kind of acute leukemia involved with myeloid cell lines and lymphoid cell lines. AMLL was not the same as Ly+AML and My+ALL, which was produced with unique clinical and biological characteristics^[3]. Some scholars believe that it may arise from pluripotent hematopoietic stem cells. But up to now, the mechanism is not clear^[4]. The incidence of AMLL is reported 1% to 7% of acute leukemia^[5], and some report even more than 20%. The incidence of AMLL in our series was 7.57%, which is in agreement with the literature. The cellular morphology of AMLL was AML and AML-M2 was the most encountered type, while M4 and M5 were rare. When the cellular performance of AMLL was ALL, L2 was the most observed leukemia. In our patients, 3 cases were AML M2, accounting for 50%; 3 cases were ALL-L2, accounting for 75%, which is in accordance with the literature^[6]. The common clinical characteristics of AMLL were older age, higher count of white blood cell, obvious anemia, bone pain, enlarged spleen and lymph nodes, and infiltration of central nervous system and kidney^[7]. However, the clinical features of AMLL in our series were not consistent with the above

reports, which could be because of the younger age of our patients.

The morphology of AMLL was mostly illegible, to be misdiagnosed as AML or ALL. In recent years, with the wide application of flow cytometry, immunophenotyping has been the principal factor to diagnose the AMLL. According to different lineages, AMLL can be divided into three types: (1) biphenotypic type: the expression of myeloid antigen and lymphoid antigen in bone marrow; (2) biclonal type; (3) bilineal type^[8]. Lee et al^[9] reported 18 cases of AMLL, with B/M in 14 cases, T/M in 3, and T/B in 1 case. CyCD22, CD10, CD19, CD2, CD3, CD7, CD33 and CD13, had the highest antigen expression while early antigens of CD34 and HLA-DR were also expressed. The children in our series were all pairs of phenotype B-Ly+/My+ biphenotypic in 8 cases, T-Ly+/My+ biphenotypic in 2 cases, and T+B-Ly+/My+ biphenotypic in 4 cases. In 14 AMLL cases, CD13 and CD14 had the highest expression of myeloid immune phenotype. CD19 and CD10 were the highest expression of B lineage expression. Of the 14 cases, 2 cases had positive expression of hematopoietic stem cell marker CD34 antigen and 7 cases had expression of HLA-DR, which is consistent with those reported in the literature.

Cytogenetic changes of childhood leukemia are recognized as an independent prognostic factor, so more recent researches have studied childhood AMLL cytogenetic characteristics^[10]. The majority confirmed that 60% to 80% of cases had cloned chromosomal abnormalities in AMLL. Sub diploid, hyper diploid, partial trisomy and the monomer body were abnormal chromosomes. In this series, 2 cases had abnormal chromosome numbers as hyper diploids, with del (15) in each one separately. Structural chromosome abnormalities t(9; 22), t(1; 9), t(9; 11), t(6; 14), t(8; 21), inv(16), t(9; 22) and t(9; 11) are more common. The abnormal structures in this series were t (9; 22), inv(16), t(1, 22)(q44, q13), t(8, 21)(q2, q22), in 3 cases. As we know, MLL gene is located on 11q23. There are many AMLL cases of the 11q23 abnormal chromosome structures causing MLL genetic changes. In addition, the emergence of Ph chromosome is bound to affect the abnormal expression of ABL gene. Ph chromosome and MLL gene rearrangements are the two poor prognostic factors^[11]. For the higher probability of Ph

chromosome and MLL in AMLL, cytogenetics and molecular genetics could also explain the poorer prognosis of AMLL. But the biomarkers of AMLL in chromosomes and genetic abnormalities have not been found. In our patients, fusion genes were detected in 5 cases, and the positive results were AMLx5/ETO, AML-ETO-positive and MLL gene rearrangements.

At present, many studies have reported the treatment of AMLL with various outcomes. Faber and Armstrong^[12] reported that CR rate of AMLL was 16.67%, while Legrand's^[13] was 47.0% and both were lower than the CR rate of AML (62%) and ALL (82%) at the same period significantly. In this article, 9 cases have received the treatment of AMLL, 4 cases were given the regimen of ANLL and 5 cases received ALL chemotherapy. After the first course of treatment, 6 patients have achieved CR; CR rate was 66.67%, which was lower than Zheng's^[14] report (75%). The reason may be related to younger age of the patients, and fewer numbers of observed cases during this short time. The limitation of the study is little case number and no follow-up.

Conclusion

Although there were many questions in the diagnosis and treatment of AMLL, these results of the study provide a basis for prevention and treatment of AMLL in children. To improve the diagnosis and treatment of AMLL, it is necessary that more researchers should collaborate and exchange their views.

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Conflict of Interest: None

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