血液疾病专栏·疑难病研究

Laboratory study on near-tetraploid acute myelogenous leukemia of childhood

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Abstract: Near-tetraploidy is a rare cytogenetic abnormality in myelocytic malignancies in children, and its significance is unknown. To investigate the characteristics of near-tetraploidy in a child with acute myelogenous leukemia (AML-M4), bone marrow smears were prepared for morphological analysis. Bone marrow samples were collected for flow cytometry, and prepared by short-term (24 hrs) unstimulated culture and R-banding for conventional cytogenetic assay. In this case, the morphological analysis of bone marrow cells showed large and prominent nuclei. The chromosomal analysis (R-banding) demonstrated a near-tetraploidy. Combined with morphological and immunophenotypic results, AML-M4 was confirmed. The patient was given four cycles of chemotherapy, and finally achieved clinical remission. However, the duration achieving the remission in the child was longer than AML children with normal karyotype. It is believed that near-tetraploid karyotype may have a great significance to the therapy and prognosis.

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Key words: Near-tetraploidy; Acute myelogenous leukemia; Prognosis; Child

儿童近四倍体急性髓系白血病的实验室研究

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[**摘 要**] 近四倍体在儿童髓系恶性肿瘤中是一种少见的遗传学异常,其意义还不清楚。该文就 1 例近四倍体的儿童急性髓系白血病(AML-M4)分析其特点。采用骨髓涂片方法分析骨髓细胞形态,收集骨髓标本做流式细胞术分析,24 h 培养 R 显带做常规核型分析。该病例骨髓细胞形态学分析显示大而突出的胞核,染色体分析显示近四倍体核型,结合骨髓形态和免疫分型结果诊断为 AML-M4。患儿经过 4 个疗程的化疗治疗,最终获得了临床缓解,但该患儿达到缓解的时间比正常核型患儿要长。我们认为近四倍体核型是影响儿童 AML 治疗与预后的重要因素。

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[**关 键 词**] 近四倍体;急性髓细胞白血病;预后;儿童

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A large number of recurring structural and numerical cytogenetic abnormalities have been described in acute leukemia. Tetraploidy or near-tetraploidy is a rare cytogenetic abnormality in myelocytic malignancies, especially in pediatric cases, and its significance is unknown. Tetraploidy in acute myelogenous leukemia (AML) is seen primarily in elderly male patients and is associated with a low remission rate and short survival[1]. In this report, the laboratory diagnosis characteristics of a girl with near-tetraploid AML is described.

Methods

Patient

A twelve-year-old girl was first seen on June 10, 2008 with a history of fever for 3 days. On physical examination, pin-point like petechia were found on both ankles. Neither pallor on skin or mucus, nor peripheral lymphoadenopathy were found. Sternum tenderness was present. On palpation, splenomegaly was found, which appeared hard and tender. Hemogram showed

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92 g/L hemoglobin, 50.7×10^9/L WBC and 92×10^9/L platelets. A diagnosis of AML-M4 was made according to the bone marrow findings. Immunophenotyping study was suggestive of AML. Cytogenetic analysis showed a karyotype of 92-95, XXXX, +8, +21, +Mar[22]/46, XX[3], including 92[2], 94[4] and 95[16]. Consequently, the clinical diagnosis of AML-M4 was established. The patient was given chemotherapy (MAVm protocol): mitozantrone 8 mg/m^2/d × 3 days (days 3-5), AraC 100 mg/m^2/d × 8 days (days 1-8), Vumon (teniposide) 100 mg/m^2/d × 3 days (days 6-8). After four cycles of chemotherapy, the child achieved clinical remission (Hemogram showed 92 g/L hemoglobin, 4.1×10^9 g/L WBC and 220×10^9 g/L platelets; Bone marrow aspirate revealed 3.5% blast infiltration). The patient's condition remains stable till present.

Bone marrow morphological examination

Bone marrow smears were made directly after sterile aspiration of the bone marrow (0.5 mL). The smears were simultaneously stained with Romanowsky-Giemsa method and cellular chemical staining including peroxidase (POX), α-Naphthol Acetate Esterase with NaF inhibition test (α-NAE + NaF) and periodic acid-schiff's reaction (PAS). AML was classified according to FAB classification and the domestic classification method (standard classification).

Immunophenotype analysis

Indirect immunofluorescence was done with FACS Calibur flowcytometer (BD Corp. US) and a panel of monoclonal antibodies against surface antigens of neoplastic cells. CD45-SSC software was used for calculating percentage of neoplastic cells with positive expression of the antigens. Auto-cells without addition of monoclonal antibodies were used as negative control. Cells were considered positive if intensity of the fluorescence was 20% or higher than the control cells. Fluorescence-labeled monoclonal antibodies included CD45, the marker of hematopoietic stem and progenitor cells (CD34 and HLA-DR), myeloid antigens (CD13, CD33, CD14, CD15, CD64, CD117 and CyMPO), B lineage antigen (CD10, CD19, CD38 and CyCD79a) and T lineage antigen (CD7, CD2).

Karyotype analysis

Chromosome specimens were prepared by sterile aspiration of bone marrow (1-3 mL). The aspirated bone marrow was either directly cultured or cultured for 24 hours before chromosome preparation, and RHG-banding was made. In each case, 25 cells at meta-mitosis phase were analyzed. The analysis was done with Cytovision Karyotypic Analysis System (AI Corp, US). Karyotype abnormality was identified and described according to the International System for Human Cytogenetic Normenclature (ISCN) (2005).

DNA content determination

DNA content measurement in bone marrow cells was performed on the FACS Calibur flowcytometer (BD Corp. US), and the results were expressed as the DNA index (ratio of DNA content in leukemic G0/G1 cells to that in normal diploid G0/G1 cells). The reference range of DNA index is from 0.9 to 1.1.

Results

Morphological analysis of bone marrow cells

Abnormal hyperplasia of myelocytic series was noted with myeloblasts and promyelocytes amounting to 33.6%. The cells were round in shape, and they had little cytoplasm, which were light-blue in color, and had plentiful tiny and uniform granules. Their nuclei were large and bizarre in shape. See Figure 1. Abnormal hyperplasia of monocytic series was noted with monoblasts and premonocytes accounting for 62.4%. Erythroid and megakaryocytic series were significantly inhibited. Cytochemical staining showed that most of the cells were POX weak-positive; NAE was positive in most of the cells, and positive reaction of some cells was inhibited by NaF; PAS was positive in part of the cells, and presenting fine particles. The possible diagnosis of AML-M4 was made according to the bone marrow examination.

Immunophenotype analysis

Neoplastic myelogenous cells accounted for 64.3%. CD34 was expressed in 82.6% of the cells, MPO 61.5%, CD13 93.7%, CD33 48.6%, HLA-DR 90.7%, CD38 37.3%, CD117 37.0%, CD64 15.2%, CD15 16.1%, and CD14 13.5%. Immunophenotype analysis showed the diagnosis of AML.

Karyotype analysis

Among the 25 cells at meta-mitosis phase, 22 had 92-95, XXXX, +8, +21, +Mar and the other 3 had normal karyotype (46, XX). The result showed a near-tetraploid karyotype (Figures 2 and 3).

DNA content determination

The DNA histogram showed double peaks. One indicated a diploid line with the DNA index of 1.0, while another represented a near-tetraploid line with the DNA index of 2.03.

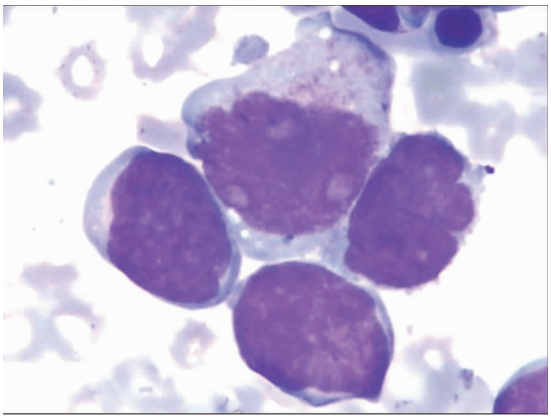

Figure 1 Morphological features of leukemic cells Nuclei were large and bizarre in shape (Wright-Giemsa staining, ×1 000).

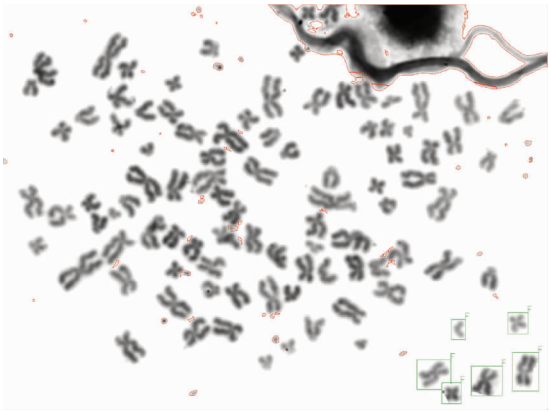

Figure 2 Karyotype analysis Near-tetraploidy was noted.

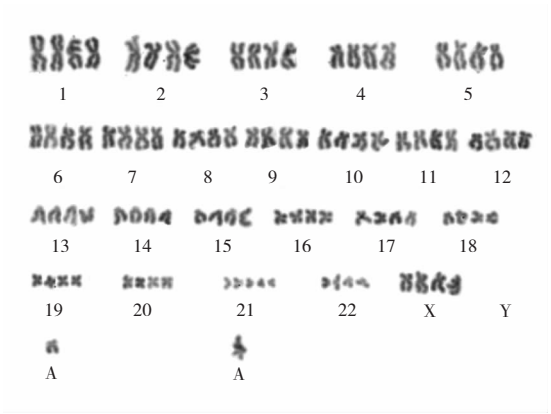

Figure 3 Karyotype of the patient Of the 25 cells at metamitosis phase, 22 had 92-95, XXXX, +8, +21, +Mar (R-banding).

Discussion

Tetraploid or polyploid metaphases were found in <2% of metaphases in normal bone marrows, most of them originating from cells of megakaryocytic lineage[2]. Tetraploidy can be found in many kinds of tumors, such as esophageal carcinoma, gastric carcinoma, carcinoma of colon and lymphadenoma. Tetraploidy was also found in acute leukemia and myelodysplastic syndrome (MDS). Hyperdiploidy was present in 14%-27% of cases of childhood ALL and 4%-9% of cases of adult ALL, and tetraploidy or near-tetraploidy accounting for 1%-2% of them[3]. Cases of tetraploid AML were less than those in ALL[4-6]. They were previously reported in FAB subtypes of M0 to M7. Association of tetraploidy with leukemia-specific translocations, especially t(8;21), has also been reported. Morphologically, tetraploidy is associated with large and bizarre blasts, probably due to the increased nucleic acid content[7,8]. In general, tumors with tetraploidy have a great malignant degree and a short survival period. Hyperdiploid ALL is sensitive to chemotherapy and has a good prognosis. Raimondi et al[9] reported that the prognosis of patients with triploid or tetraploid ALL was not significantly different from that of patients with hyperdiploid ALL. The adult patients with tetraploid AML were not sensitive to chemotherapy, and had a low remission rate and a short survival period[5,6].

The case in this study was a 12-year-old girl, and the final diagnosis was AML-M4. The blast cells were large, with a low nuclear/cytoplasmic ratio, bizarre nuclear configuration, and multiple nucleoli. The cytoplasms were light-blue in color, and had plentiful tiny and uniform granules, but Auer rods were rarely seen. This kind of nuclei cannot be found in some cases with non-tetraploid AML. So it was thought that the occurrence of the giant and bizarre blasts were morphological features of tetraploid or near-tetraploid leukemia. Karyotype analysis was thus suggested to detect tetraploid or near-tetraploid metaphases in patients with giant and bizarre blasts displayed on bone marrow smears. Near-tetraploidy has ever been found in AML-M2 patients, and it is essential to distinguish AML-M4 from AML-M2 in morphology [10]. Dinçol et al[11] found a case of near-tetraploid acute mixed leukemia. In that case, both myeloid and NK cells in leukemic cells were found by immunophenotyping and FISH analysis. Combined use of multiple methods such as immunophenotyping, FISH and karyotype analysis are helpful in the accurate diagnosis of leukemia.

Cytochemical staining and flow cytometric immunophenotype analysis in this patient did not show significant differences from AML-M4 patients with normal karyotype. However, there were differences in the DNA content determination between them. The DNA index (2.03) in this patient was obviously higher than that in patients with normal karyotype (0.9-1.1).

Tetraploid clones were verified in this patient. This indicates DNA index has a great significance in the diagnosis, therapy and prognosis of tetraploid AML in children.

Clinically, with the same conventional chemotherapy regimen, the child in this study showed low sensitivities to treatment compared to AML-M4 children with normal karyotype. Although she achieved a clinical remission finally, the duration achieving the remission was longer than other children with normal karyotype. This might be owing to the underlying multiple karyotypic aberrations[6]. Abnormal tetraploid karyotypes involved may contribute to the laboratory and clinical features, and have a great significance to the therapy and prognosis.

The AML case with tetraploid or near-tetraploid karyotypes is rarely seen in children. The studies on the laboratory and clinical features of this disorder are limited. The diagnosis of this case was based on evidence of laboratory findings that included karyotype analysis, immunophenotype analysis and DNA content determination. These methods provided more convincing evidence for the tentative diagnosis based on the morphological observation. This also indicated good feasibility of MICM classification diagnosis for leukemia. This research might provide new thoughts in clinical management of multiploid AML in children, i. e, an individual chemotherapy regimen may be proposed combined with the morphological, immunophenotype and cytogenetic features.

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