Flow Cytometry DNA Measurement for Prediction of Effect of Therapy in Acute Leukemia

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Summary: We measured the DNA content of bone marrow (BM) aspirate by flow cytometry (FCM) in 41 cases of acute leukemia (AL) and found no remarkable relation between pretreatment DNA index and percentage of proliferative cells and prognosis (P > 0.05). After chemotherapy, reduction of proliferative cells rate, disappearance of DNA aneuploidy and decrease of leukemic cells in BM could be considered as the reliable indicators of effective treatment. Complete remission (CR) rate was significantly higher in patients responsive to treatment (81.82 %) than in those unresponsive (22.22 %; P < 0.05). It seems that these parameters may serve as indicators of efficiency of chemotherapy and be useful in monitoring regimen in time and in reducing the occurrence of chemotherapy-resistance, thereby facilitating individualization of chemotherapy in AL patients.

Key words: DNA measurement, flow cytometry, acute leukemia, chemotherapy efficiency

Examination of bone marrow (BM) is a common method for diagnosing and classifying acute leukemia (AL). However, it is difficult for this method to predict chemotherapy efficiency and prognosis of the disease. By use of flow cytometry (FCM) it is possible to investigate various cellular biochemical parameters of multitude of cells quantitatively, quickly and accurately. Using FCM, a number of investigators abroad have made encouraging progress in the study of classification of AL, efficiency of chemotherapy and prognosis of the disease. To our knowledge, no such study has been reported at home. DNA content of BM aspirate from 41 cases of AL was measured by FCM to evaluate its significance in judging chemotherapy efficiency and prognosis.

MATERIALS AND METHODS
1. Clinical data

41 cases of AL (37 newly diagnosed and 4 relapsing cases) were diagnosed by BM cell morphology and histochemical staining. According to FAB classification, there were 11 cases of acute lymphoblastic leukemia (ALL, 5 L1, 3 L2, 2 relapsing L1, 1 lymphosarcoma cell leukemia) and 30 cases of acute non-lymphoblastic leukemia (ANLL; 7 M2, 11 M3, 2 relapsing M3, 2 M4, 7 Ms and 1 secondary to myelodysplastic syndrome. Considering the patients' condition, low-dose cytosine arabinoside (Ara-C) or combined chemotherapy consisting mainly of Ara-C was administered. BM examination and FCM-DNA assay were done 2 to 3 days or 7 to 8 days before and after chemotherapy. We estimated the therapeutic effect in 30 cases of AL who had one course of chemotherapy according to the criteria set up by Suzhou Symposium on Leukemia Therapy in 1987. Complete remission (CR) rate was 55.56% (5/9) in ALL and 47.62% (10/21) in ANLL.

60 cases served as controls. The specimens included 34 samples of venous blood (VB) from non-hematologic diseases, 13 BM aspirates from iron deficiency anemia and megaloblastic anemia, and 13 BM irrigates of resected ribs.

2. Methods

1) Preparation of mononuclear cell suspension

VB, BM aspirates and BM irrigations were anticoagulated with 125 U/ml of heparin or defibrinated. Mononuclear cells were obtained by separation with Ficoll Hypaque gradient centrifugation (d=1.077 g/ml, 400 g, 20 min), followed by 2 washes in normal saline, fixed in 95% ethanol and stored at 0°C.

DNA index = \[ \frac{\text{mean channel number of sample cell } G_{0/1} \text{ peak}}{\text{mean channel number of normal lymphocyte } G_{0/1} \text{ peak}} \]

In this study DNA index value from normal diploidy lymphocyte was 1 ± 0.10 (\( \bar{x} \pm 2 \sigma \)). If DNA index did not fall in or bore no multiple relation with the range, DNA aneuploidy could be considered. More than one cell

2) Staining for DNA analysis

Ethidium bromide single step staining method was used. The staining solution contained 10 µg/ml ethidium bromide, 10 µg/ml ribonuclease and 1% triton-x-100. The fixative was washed off before test. The cell count was adjusted to finally \( 3-5 \times 10^6/\text{ml} \). One drop of red blood cell (RBC) of chicken and 1 ml ethidium bromide staining solution were added for DNA stain. The sample was measured after 30 min at 4°C.

3) Measurement of DNA content

A fluorescence activated cell sorter (FACS-420, manufactured by Becton Dickinson Co., USA) was used for DNA measurement. A 488-nm argon ion laser line at 300 milliwatt acted as stimulating source. The fluorescence of cellular DNA was amplified by photomultiplier, recorded by multipulse analyser and visualized on the screen as histogram and numeration table. DNA content and cell cycles were calculated. In order to ensure the accuracy and stability of the device, the comparability and reproducibility of the test and to reduce the possibility of abnormal DNA findings caused by test-induced error, a standard model was checked up before each assay. The standard model consisted of suspension of chicken RBC and of normal human lymphocytes with the chicken RBC peak kept at channel 20 and the coefficient variation (CV) at about 5% (CV = 0.425).

\[ \text{mean} \quad \frac{\text{fall width at half maximum}}{\text{mean}} \quad (44). \quad 1.5-3 \times 10^4 \text{ cells were analyzed in each sample.} \]

3. Laboratory data analysis

1) DNA index was expressed as the relative DNA content of the cell calculated by the formula

\[ \frac{\text{mean channel number of sample cell } G_{0/1} \text{ peak}}{\text{mean channel number of normal lymphocyte } G_{0/1} \text{ peak}} \]

In this study DNA index value from normal diploidy lymphocyte was 1.0 ± 0.10 (\( \bar{x} \pm 2 \sigma \)). If DNA index did not fall in or bore no multiple relation with the range, DNA aneuploidy could be considered. More than one cell