Morphological and immunological criteria of minimal residual disease detection in children with B-cell precursors acute lymphoblastic leukemia

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Abstract. One of the key factors of prognosis and risk stratification in patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is minimal residual disease (MRD). Identification of MRD on the day 15th is one of the most significant in prognosis of the disease. We compared data of a morphological and flow cytometry results of assessment of a bone marrow (BM) at the day 15th of induction chemotherapy in children with BCP-ALL.

1. Introduction

Detection of minimal residual disease (MRD) in bone marrow (BM) in children with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is one of the key factor of prognosis and at each step of the therapy it is solved particular clinical task [1].

Long time the assessment of therapy efficacy was based on a morphological method – light microscopy of a bone marrow sample stained by Romanovsky-Gimze method with counting percentage of blast cells.

According to morphological criteria on the level of blasts allocates M-variants: M1 - <5% of blast cells, 5-25% - M2 and more then 25% of blast cell – M3 [3]. Despite the relative simplicity of execution and economic appeal of this method, it's sensitivity is low (up to 102). Also certain difficulties is made by a mimicry of blasts and impossibility to distinguish them from the regenerating BM precursors on later terms of MRD monitoring that reveals limitation of morphological approach, and needed of search of alternative techniques.

Multicenter international research works proved clinical significance of immunological MRD detection by flow cytometry (FC) [4]. Morden FC has comparable sensitivity with molecular methods due to the opportunity of assessment up to 12 parameters at each cell and analyzing millions of cells at the same time [9]. Now monitoring of MRD baised on detailed charachteristics of blast cells immunophenotype and selection of optimal combinations of antigens at the stage of diagnosis [10].
One of the most significant time point in MRD detection is 15th day of induction chemotherapy, with reflects a primary answer of tumor to treatment [4-6]. Taking into account the lack of normal BCP in BM at the day 15 [4, 5] FC strategy at this time point is to detect CD10+BCP in case of pre-pre-B immunosubtype. Earlier, we proved reliable comparability of 3- and 4-5 colors FC in MRD detection at the day 15.

2. Materials and methods

In a research were enrolled 130 children (64 boys and 66 girls) with BCP-ALL, whom MRD were detected at the day 15 of induction chemotherapy. Middle age was 5.5 (1-17 years). Pre-pre-B immunosubtype was diagnosed in 92.3%, pro-B – 7.7%. Patients were treated according to ALL IC BFM 2002 (40 of 130) and ALL IC BFM 2009 (90 of 130) protocols. In all cases morphological and immunological tests were provided from one tube with BM aspirate. In 120 cases MRD detection was with 3-5 colors FC protocol. In 8 cases were use 8-color EuroFlow FC protocol.

Immunological assessment of MRD was based on detection of CD10 and CD34 expression and CD45 dim expression or its lack in a gate of CD19+ cells. In all cases to exclude debris and to simplify calculation was used Syto-family dye (Syto16 or Syto41).

The algorithm of a gating is presented at figure 1.

Figure 1. The algorithm of gating in MRD detection at the day 15 of induction chemotherapy. At the cytogramm A performed calculation of nucleated cells according to the expression Syto16 (x axis) vs side scatter parameters (y axis) – gate 1, red. Syto16+ 91.65%. Cytogramm B performed detection of CD19+ cells 11.81% (CD19 – x axis vs side scatter parameters (y axis) – gate 2, blue. Cytogramm C – 2.52% CD10+BCP detected by expression of CD10 antigen (y axis) vs CD19 (x axis), gate 3, green. Cytogramm D – 2.35% CD34+ BCP detected by expression CD34 antigen (y axis) vs CD19 (x axis), gate 3, green.
In case of pro-B ALL immunosubtype MRD detection based on calculation of TdT+cyCD22+BCP (figure 2).

**Figure 2.** MRD detection by expression of TdT and cyCD22 antigens in case of pro-B ALL. Cytogramm A – calculation of CD19+ cells (CD19 – x axis vs SSC parameters – y axis) gate 2 – 5.5%, blue. Cytogramm B – 0.03% of BCP detected (TdT y axis vs cyCD22 x axis), gate 3, green.

3. Results

Samples were analyzed according to criteria of M-variants. M1 group was made by 80% of samples, M2 group – 14.6%. In M3 group was 5.4% of samples. In each group the quantity of blast and lymphocytes, cellularity of samples, and also the MRD levels were compared. Morphological criteria are compared with immunological data. Each group of samples is in details analyzed concerning the MRD levels according to criteria of stratification of the BFM protocol: 0.1% - standard risk, 0.1-10.0% - intermediate risk and 10.0% and more – high risk group. Standard threshold level of MRD-negativity is 0.01% of tumor cells in a sample.

M1 group samples (104 samples) was characterized by the higher percent of lymphocytes (28.0%). Presence of MRD-positive samples (FC) at the M1 group (group of the good morphological answer) can be explained with a mimicry the blast cells, that is their full morphological similarity with lymphocytes. It complicates a possibility of accurate morphological verification of residual leukemic blasts.

M2 group was made by 19 BM aspirates. BM samples with the maintenance of MRD cells less than 0.1% (standard risk) according to FC in this group were absent. Morphological and FC data and the M2 group, didn't contradict each other.

In M3 group was made by 7 samples. In the group there was no samples with quantity of MRD cells less than 1.0% according to a FC. In 85.7% of samples of this group (6 of 7) on data of FC has revealed 10.0% and more MRD-positive cells.

By comparison of average values of indicators of the analyzed groups reliable distinction in number of lymphocytes is established when comparing the M1 and M3 groups (p <0.0001) and the M2 and M3 groups (p = <0.0001).

4. Conclusion

Thus, from the carried-out work it is possible to draw the following conclusions.

At the MRD monitoring it is necessary to provide morphological and immunological tests using BM aspirate of one tube with obligatory calculation of sample cellularity.
Morphological and FC data are coincide. In the M2 and M3 groups, immunological MRD-negative samples aren't revealed. In the M1 group the majority of samples were MRD-positive according to FC, 42.9% from them contained no more than 0.1% of MRD cells.

Presence the MRD-positive samples at M1 group can be explained with a mimicry the blast cells, that is their similarity with lymphocytes that complicates accurate morphological verification of blasts.

FC, having higher sensitivity in comparison with a morphological method, allows to stratify more precisely patients on risk groups (at the M1 group there were samples both from group standard and intermediate and from group of high risk).

Introduction to the panel of MRD detection the dyes of the Syto-family is obligatory. It allows to allocate accurately nucleated cells of sample, simplifies recalculation of quantity of MRD cells and gives the chance to exclude debris from the analysis. Dilution of a sample by debris can lead to understating of quantity of MRD cells and as a result – incorrect risk stratification of patients.

Acknowledgments
This work was supported by Competitiveness Program of National Research Nuclear University MEPhI.

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