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Significance of CD34/CD123 expression in detection of minimal residual disease in B-ACUTE lymphoblastic leukemia in children

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ABSTRACT

Background: MRD is seen as the major cause of disease relapse. So, it gives important feedback about conventional treatment success and helps in selecting therapeutic alternatives. We aimed to compare the expression of CD34/CD123 on normal B-cell precursors in bone marrow ("hematogones") and on leukemic blasts in B-acute lymphoblastic leukemias (B-ALL) pediatric cases by flowcytometric analysis. Our study conducted on 20 children as a control and 30 B-ALL children cases at diagnosis and after 28 days of induction therapy. We found that the less mature hematogones (dim CD45+) that express CD34 lack CD123 expression, whereas the more mature hematogones (moderate CD45+) lack CD34 but always express CD123. In contrast with this discordant pattern of CD34 and CD123 expression in hematogones, blasts in 24 of 30 cases (80%) of B-ALL showed concordant expression pattern of the 2 antigens: 63% (19 of 30) cases expressed both antigens, whereas 17% (5 of 30) expressed neither. Our study concluded that these distinct patterns of CD34/CD123 expression on hematogones (discordant) and B-ALL blasts (concordant) are useful in differentiating small populations of residual blasts from hematogones after induction therapy to detect MRD.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for approximately 20% of all cancers and 75% of all leukemias among patients younger than 20 years of age [1]. It is an aggressive but potentially curable disease in which monitoring the immediate and early response to therapy is of critical importance for optimal management [2]. Current management protocols require assessment of residual leukemic cells at defined intervals after initiation of chemotherapy. Increased numbers of hematogones are often present in these specimens and may cause problems in interpretation because they share many morphologic and immunophenotypic features with neoplastic lymphoblasts of B-ALL. A flow cytometric test that can reliably separate hematogones from B-ALL would be helpful in cases of suspected recurrent B-ALL [2].

IL-3 stimulates cell cycle progression in early hematopoietic progenitors and promotes differentiation in a broad spectrum of hematopoietic cells, including pre-B and pro-B cells, in concert with other growth factors [3]. CD123 is the α chain of the human interleukin (IL)-3 receptor and is essential for the formation of the high-affinity heterodimeric IL-3 receptor [4] IL-3R can be widely expressed in the blood malignancies. Activation of cytokine receptor affects the hematopoietic cell survival, proliferation and differentiation [5].

Our study aimed to characterize the expression of CD34/CD123 in normal B-cell precursors (hematogones) in the bone marrow in comparison to leukemic blasts of B-ALL and distinguished residual leukemic blasts from hematogones for monitoring the early response to therapy which is of critical importance for optimal management.

2. Materials and methods

2.1. Patients and samples

This study conducted on 30 pediatric patients with B-ALL initially at diagnosis and after day 28 of induction therapy (only 23 cases of them, as the others were not available). We also selected 20 children with matched age, sex and non-malignant bone marrow to act as a control group. Age of ALL patients ranged from 0.25–15 years (Mean age ± SE, 5.3 ± 0.6), they comprised of 14 males (46.7%) and 16 females (53.3%). The control subjects were of different diagnoses; 14 ITP (70%), 2 reactive bone marrow (10%), 1 eosinophilia (5%), 1 erythroid hyperplasia (5%), 1 bicytopenia (5%) and 1 case suffered from fever of unknown origin (5%). All groups were selected from Benha Children Hospital (BENCH) after taking approval from their parents and subjected to complete medical history, physical examination and laboratory investigations including complete blood count (by Medonic M-20, Stockholm-

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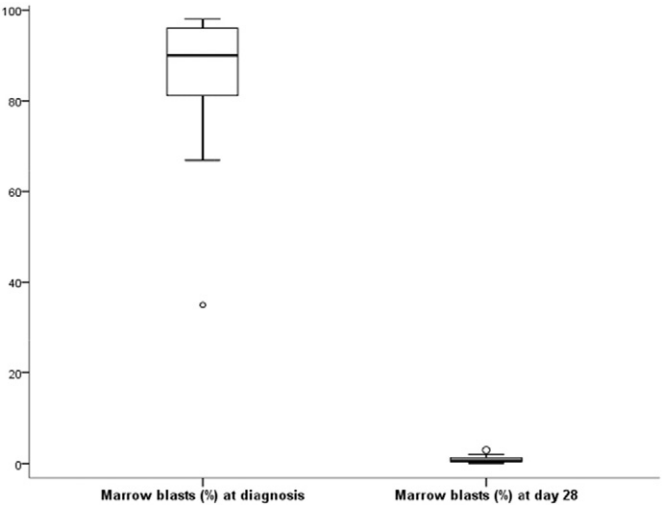

Fig. 1. Marrow blast percentage at diagnosis and at day 28.

Sweden), bone marrow examination and immunophenotypic classification. One milliliter of B.M. sample was collected from each selected case and control into a tube containing EDTA for immunophenotyping and flowcytometric analysis of CD34 and CD123 expression. B.M. samples were collected from B-ALL patients initially at diagnosis and at day 28 of induction therapy.

2.2. Flow cytometric analysis

2.2.1. Reagents supplied

Fluorochrome-conjugated antibodies to the following antigens were used to profile hematogones and B-ALL cells at indicated quantities per test: fluorescein isothiocyanate (FITC)-conjugated isotype control antibodies for IgG1, CD34. Phycoerythrin (PE) - conjugated isotype control antibodies for IgG2a, CD10 and CD123 were used at 10 μL per test. CD45 and CD19 were labeled with peridinin chlorophyll protein–cyanine (Per-CP) and used at 10 μL per test (All monoclonal antibodies were purchased from Becton Dickinson, San Jose, CA).These antibodies were used in combination (cocktails) to form 2 or 3-color panels. The following combinations were used for hematogone analysis, with the antibodies in each combination being conjugated with FITC, PE and Per-CP (1) isotype control IgG1 (FITC)/IgG2a (PE) (2) CD10 (PE) and CD19 (Per-CP) (3) CD34 (FITC), CD123 (PE) and CD45 (Per-CP).Additional combinations used for analysis of B-ALL cases to classify them immunophenotypically and examine myeloid antigen expression for CD33.

2.2.2. Staining and acquisition

Staining was performed using the 2 or 3 color combinations of the conjugated antibodies listed in the preceding section by adding 10 μL of monoclonal antibody on B.M. sample and incubating tubes for 20 min in the dark at room temperature. Then, RBCs were lysed using FACS Lyse solution (Becton Dickinson) for 10 min and centrifuged at 1200 rpm for 5 min. The supernatant was aspirated and the pellet was resuspended and washed with 2.0 mL of phosphate-buffered saline (PBS) twice before being resuspended in 0.5 mL of PBS and examined. An isotype-matched negative control sample (BD Biosciences, San Jose, CA) was used in all cases to assess background fluorescence intensity.

Stained cells were acquired on a FACS Calibur flow cytometer (BD Biosciences) that was set up using validated quality assurance procedures. At least 10,000 events were acquired for cases at diagnosis and 50,000 events were acquired for cases at day 28 and control group.

Data Analysis:

The data were analyzed using the CellQuest software program (BD Biosciences). The following gating strategies were used in control group for analysis of hematogones and mature B lymphocytes. First: hematogones; beside there were positive for CD10 and CD19; they were identified by their low side scatter and variable CD45 (dim to moderate) and divided into 2 groups. The first group comprised less mature hematogones that expressed CD34 and had dim CD45.The second group was composed of more mature hematogones lacking CD34 but with moderate CD45 expression. CD123 was examined in relation to CD34 in both groups of hematogones. N·B. Hematogones had to be identified to be distinguished from blast cells, as it morphologically similar to blast cells. So after treatment; any cells with different pattern of hematogones; considered as blast cells (MRD). Second: Mature B lymphocytes; beside they are positive for CD19 and CD20; they were identified by their specific side scatter and bright CD45 and examined for CD123 in relation to CD34. So, CD123 expression in relation to CD34 were assessed in (dim, moderate and high CD45) to assess pattern of expression.

In B-ALL pediatric cases; an inclusion gate (G1) was first set on viable blast cells based on forward light scatter and side light scatter. An isotype control was used for quadrant adjustment to subtract autofluorescence and nonspecific binding. In cases of B-ALL, at diagnosis, the dominant population of leukemic blasts was identified in the CD45-vs-side scatter histogram (G2), and the expression of CD34 and CD123 on this population was then assessed (CD34-vs-CD45, CD123-

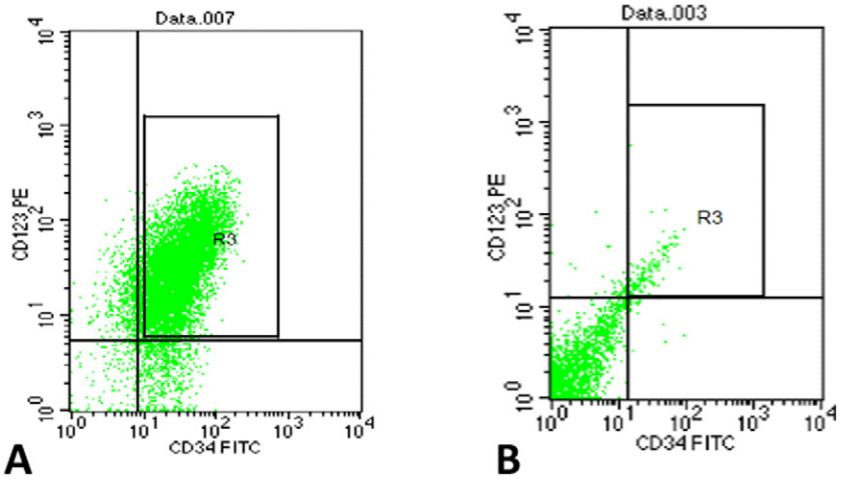

Fig. 2. A- show double positive population of CD34/CD123(R3) on leukemic blasts at diagnosis of B- ALL cases. B- show residual leukemic blasts at day 28 [CD34+/CD123+](R3).