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.

Key words: CD34; CD123; B-ALL; Minimal residual disease; IL3 receptor.

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. 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. 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.
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. 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. IL-3R can be widely expressed in the blood malignancies. Activation of cytokine receptor affects the hematopoietic cell survival, proliferation and differentiation.

**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.

**Materials and methods:**

**I - 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-Sweden), bone marrow examination and immunophenotypic classification. One ml 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.

**II-Flow cytometric analysis:**

**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 perdinin 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.
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 minutes in the dark at room temperature. Then, RBCs were lysed using FACS Lyse solution (Becton Dickinson) for 10 minutes and centrifuged at 1,200 rpm for 5 minutes. 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 auto-fluorescence 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-vs-CD45 and CD34-vs-CD123) according to this gate. The double positive population for CD34 and CD123 then gated (G3).

At day 28 of induction treatment, the cell population with dim to moderate expression of CD45 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-vs-CD45 and CD34-vs-CD123) according to this gate. The double positive population for CD34 and CD123 was identified by applying G3 gated at diagnosis on the CD34-vs-CD123 histogram at day 28 of induction treatment.

III-Statistical Analysis:

The statistical analysis of data was done by using excel (Microsoft Office program, 2010) and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Kolmogorov-
Smirnov test was done to test the normality of data distribution. Qualitative data were presented as frequency and percentage. Chi square and Fisher exact tests were used to compare groups. Quantitative data were presented as median, range, mean and standard error (SE). For comparison between two groups; Mann-Whitney test (for non-parametric data) was used. For comparison between more than two groups; Kruskal Wallis (for non-parametric data) was used. Changes in numerical variables over time were examined by the Wilcoxon signed rank sum test. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. N.B: p is significant if it is ≤0.05 at confidence interval 95%.

**Results:**

In our study, there were significant decrease in TLC, blast percentage and significant increase in hemoglobin concentration and platelet count at day 28 when compared to same parameters at diagnosis, *Fig (1).*

There were no significant statistical difference between each of the age, gender, laboratory data and cytogenetic status in different CD34/CD123 patterns of expression; at diagnosis and at day 28 of studied ALL cases. There were no significant differences between common B and pre-B immunophenotypes in different CD34/CD123 patterns of expression; at diagnosis and at day 28. There was significant increase in CD34 expression on ALL blasts when compared to hematogones with moderate CD45 in control. There was no significant difference between CD34 expression on ALL blasts when compared to hematogones with dim CD45. There were significant differences in CD34 positivity of ALL blasts when compared to hematogones with either dim or moderate CD45. There was significant increase in CD123 expression on ALL blasts when compared to hematogones with either dim or moderate CD45. Also there was significant difference between CD123 positivity; on ALL blasts when compared to hematogones with dim CD45 expression. There was no significant difference between CD123 positivity; on ALL blasts when compared to hematogones with moderate CD45 expression. Also there were significant differences between CD34/CD123 patterns of expression in ALL blasts when compared to control; on each of the dim and moderate CD45.

The concordant expression pattern of CD34 and CD123 in B-ALL blasts differs from the discordant expression of these antigens in hematogones. This distinction is useful in detection of double positive population (CD34+/CD123+) as a residual leukemic blasts (MRD), with cutoff value of 0.1%, in the bone marrow of patients treated for B-ALL (at day 28). CD34 and CD123; expressions and positivity were decreased significantly in day 28 when compared to those measured at diagnosis. Although CD34+/CD123+ population expressions were decreased significantly in day 28 when compared to those measured at diagnosis; positivity did not reach significant level (*Table 1, 2*).

By cross tabulation of CD34/CD123 patterns of expression; at diagnosis and at day 28. Five cases were double negative at diagnosis; 3 cases remained double negative at day 28, 1 case became positive for CD34 and 1 case referred. Six cases were positive for CD123 only at diagnosis; 3 of them became double negative, 1 died in induction and 2 referred. Nineteen cases were double positive at diagnosis, 9 of them became double negative at day 28, 1 remained double positive, 4 cases became positive for CD34 only, 2 cases became positive for CD123 only, 1 case died during induction therapy and 2 cases were referred (*Table 3*).
In our studied cases, Twenty three cases achieved complete remission (CR) (92%), 2 cases died during induction therapy (8%), 5 cases relapsed after CR (21.7%) and 4 cases died (16%) during the entire period of the study (Table 4).

By studying the survival times of studied cases, mean overall survival (OS) was 28.4 months and mean disease free survival (DFS) was 29.8 months. Cumulative proportion of ALL cases surviving at 48 months was 81.1% for OS and 88.1.6% for DFS. No significant differences were found between clinical outcome in both positive and negative MRD, but all cases who relapsed and those who died during follow up had positive MRD (Table 5). Also there were no significant differences between survival times in different CD34/CD123 patterns of expression (Table 6).

Discussion:
MRD is seen as the major cause of disease relapse, its detection is at the crossroads of past and future concerns regarding hematological malignancy management. It gives important feedback about conventional treatment success and helps in selecting therapeutic alternatives.

Hematogones are the normal bone marrow precursors of mature B-lymphocytes with morphologic and immunophenotypic properties that overlap those of lymphoblasts. They were originally identified by their characteristic morphologic features in bone marrow smears. Later, their relatively distinct flow cytometric immunophenotype was identified.

Our work demonstrated that in mature B-lymphocytes with high CD45 expression, CD34 and CD123 were double negative in 4 cases (20%) and discordant in 16 cases (80%) which was highly compared with Hassanein et al study, as they found that the mature B- cells (with high CD45 expression) lacked expression of CD123 in every case without detectable hematogones, and also suggested that CD123 expression on normal mature B- cells is probably lost in time dependent manner.

Also we revealed that CD34/CD123 pattern of expression in either dim or moderate CD45 expression (hematogones) in control group; had discordant pattern of expression which was in agreement with Hassanein et al study, as they reported that CD123 expression was found to be asynchronous in relation to CD34 in both groups of hematogones (dim and moderate CD45 expression). Thus, the majority of the less mature hematogones (dim CD45) expressed CD34 but did not express CD123. On the other hand, the more mature hematogones (moderate CD45), which did not express CD34, showed expression of CD123 on most cases. Also, these findings were in agreement with Munoz et al study, which reported that CD123 was negative in the more primitive CD34+/CD38-compartment.

In our study, ALL cases at diagnosis presented different pattern of expression; 5 cases had double negative CD34/CD123 pattern of expression (16.7%), 19 cases had double positive (63.3%) and 6 cases had discordant results (20%), which were highly compared with Hassanein et al results, as they found that in 80% of cases, leukemic blasts expressed CD34 and CD123 (double positive), and in 11% of cases neither antigens was expressed (double negative) and only in 9% of cases , the blasts did not express CD34 (positive CD123 only).
The present study revealed that there was significant increase in CD123 expression on ALL blasts when compared to hematogones, also there was significant increase in CD123 positivity on ALL blasts when compared to hematogones with dim CD45 expression, which were in agreement with Djokic et al. study, which reported that the overall CD123 expression in precursor-B-ALL was significantly higher than in normal B-cells (p < 0.0001) of any maturation stage, also they reported that CD123 showed much less variation of expression than other commonly used markers for MRD including CD34, TdT and CD10.

The current study reported that there was significant difference in CD34/CD123 patterns of expression on ALL when compared to hematogones which was in agreement with Hassanein et al study, which provided that the concordant expression pattern of CD34 and CD123 in B-ALL blasts differs from the discordant expression of these antigens in hematogones.2

In the present study, CD34/CD123 expression and positivity were decreased significantly in day 28 of treatment when compared to those measured at diagnosis; which were highly compared with Djokic et al conclusion, in which they found that CD123 expression is generally low in B-cell precursors and mature B cells both in human control bone marrow samples and in post chemotherapy regenerating B-cells.

Although CD34+/CD123+ population expressions were decreased significantly in day 28 (due to the effect of therapy) when compared to those measured at diagnosis; positivity did not reach significant level because of small sample size.8

By cross tabulation of CD34 and CD123 positivity at diagnosis and at day 28, five cases were double negative at diagnosis; 3 cases remained double negative at day 28, 1 case became positive for CD34 and 1 case referred. Six cases were positive for CD123 only at diagnosis; 3 of them became double negative, 1 died in induction and 2 referred. Nineteen cases were double positive at diagnosis, 9 of them became double negative at day 28, 1 remained double positive, 4 cases became positive for CD34 only, 2 cases became positive for CD123 only, 1 case died during induction therapy and 2 cases were referred. These results were marginally compared with Djokic et al and Hassanein et al studies.2,8

Djokic et al results showed stable CD123 over expression in 77% samples with residual leukemia.8 Hassanein et al previous study found that there were residual leukemic blasts could be detected in 28 cases from 45. Of these, blasts from 27 cases were positive for CD34 and CD123 at initial diagnosis, and 1 was negative for CD34 and CD123. In each of these cases, the expression pattern of these 2 antigens remained constant after chemotherapy.2

In our study, no statistical significant differences were found in each of the age, gender, immunophenotypic classification and each laboratory parameter in different CD34/CD123 patterns of expression; at diagnosis and at day 28 in ALL cases. According to available knowledge, there was no previous work studied these relations except the work of Ge et al,5 as they found that CD123 expression is highly correlated with high leucocytes in their studied cases of elderly ALL patients. These differences may be due to small sample size and difference in age of studied cases.
In the present work, no significant differences were found in clinical outcome in different CD34/CD123 patterns of expression; at diagnosis and at day 28 and also, no significant difference were found in clinical outcome in both positive and negative MRD, but all cases who relapsed and those who died during follow up, had positive MRD (CD34+/CD123+ population).

To our knowledge, no previous work studied the relation between clinical outcome in different CD34/CD123 patterns of expression; at diagnosis and at day 28 and in both positive and negative MRD except the work of Ge et al on elderly ALL patients, which suggested that the high expression of CD123, HLA-DR, CD34 and CD117 antigens could be a high negative factor for prognosis, these results may be explained by small sample size and short period of follow up.5

**Conclusion:**
Our study concluded that there was a discordant pattern of CD34/CD123 expression on hematogones in contrast with a concordant pattern of CD34/CD123 expression on B-ALL blasts. This distinction is useful in correctly differentiating small populations of residual blasts from hematogones.

**References:**


