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Immunophenotypic Comparison between Reactive Bone Marrow B-Lymphocyte Precursor (Hematogones) and B-Neoplastic Lymphoblast Leukaemia Using Cd 34, Cd 123 by Flowcytometry

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Abstract

Background Flow cytometric study found that lymphoblasts of B acute lymphoblastic leukemia exhibited multiple aberrant antigens by which they can be distinguished from hematogones. These antigens are CD34 and CD123.

Objective To determine the immunophenotypic pattern of CD34 and CD123 expression in hematogone of reactive bone marrow and in neoplastic lymphoblast in B-acute lymphoblastic leukemia (ALL) patients and to evaluate the impact of that pattern in the residual disease detection after chemotherapy.

Methods This is a case control study to determine the expression of CD34 and CD123 in 30 patients newly diagnosed with B-ALL. Re-assessment was done for 20 patients of them after 4-6 weeks of chemotherapy; in addition to 10 patients with reactive bone marrow to assess hematogones.

Results In (93.4%) of the newly diagnosed B-ALL cases, leukemic blasts expressed both CD34 and CD123, Conversely, in (6.6%) cases, neither antigen was expressed. In hematogones; the immature hematogones (dim CD45, CD34 +) did not express CD123 while the mature hematogones (moderate CD45+, CD34-) expressed CD123. The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and hematogones respectively in post chemotherapy patients remain stable.

Conclusion The distinct pattern of CD34 and CD123 expression on hematogones (discordant) and B-ALL blasts (concordant) is useful in correctly classifying immature B cells as residual leukemic blasts or hematogones in the bone marrow of patients treated for B-ALL.

Keywords B-Acute Lymphoblastic leukemia, Flow cytometry, Immunophenotypic aberrancy, Hematogones, CD34 and CD123.

List of Abbreviations: ALL = Acute lymphoblastic leukemia, BM = Bone marrow, FAB = French-British-American, HGs = hematogones, RD = residual diseases, WBC = white blood cell count, PAS = Periodic Acid Schiff, FC = Flow cytometry, SBB = Sudan Black B.

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal hematologic disorder. It involves excessive proliferation and impaired differentiation of leukemic blasts that lead to inadequate normal hematopoiesis. Thus, patients usually present with symptoms resulting from bone marrow failure [(1)]. It comprises approximately 80% of pediatric acute leukemias and 20% of adult cases [(2)].

The non-neoplastic counterparts of leukemic B lymphoblasts, normal bone marrow B-cell precursors, are commonly referred to as hematogones [(3)]. Hematogones may be abundant in healthy infants and children and there was a significant decline in hematogones

with increasing age, but a broad range was found at all ages [3,4].

Hematogones, especially if present in large numbers, may confound the diagnosis of B-ALL in 1 of 2 ways: (1) Hematogone hyperplasia in a background of cytopenias may be mistaken for B-ALL at initial diagnosis. (2) Increased hematogones in a patient treated for B-ALL may be mistaken for residual or recurrent leukemia. By flow cytometry all cases of precursor B-lymphoblastic leukemia/lymphoma (B-ALL) demonstrate multiple immunophenotypic aberrancies relative to normal maturing B-cell precursors (hematogones) [3-5].

CD123 is the α-chain of IL-3 receptor (IL-3R), a member of the cytokine receptor super family [6]. Earlier experiments have shown that IL-3 plays an important role in the leukemogenesis of lymphoid and myeloid cells, inducing these cells to grow autonomously [7]. CD34 is a human stage-specific hematopoietic differentiation antigen, in leukemia cells; it remains expressed over several stages of lymphoid and myeloid maturation [8]. Various authors have shown that hematogones display surface staining for CD123 only in the more mature fraction that lacks CD34 expression. This “discordant” pattern is in contrast with the almost invariable “concordant” expression of these 2 antigens in B-ALL blasts [6,7,9].

While a morphologic and immunophenotypic overlap exists between hematogones and leukemic lymphoblasts, we demonstrate that morphologic review combined with evaluation of immunophenotype using (CD34, CD123) can help distinguish hematogone from leukemic blasts of B-ALL.

Methods

This case control study was conducted from November 2013 to June 2014 and included thirty patients newly diagnosed with B-acute lymphoblastic leukemia collected randomly in relation to age and gender. T- ALL and L3 were excluded from the study; Re-assessment was done for 20 patients of them after 4-6 weeks of chemotherapy (they were included according to the availability of their bone marrow aspirate BMA sample after chemotherapy). In addition, 10 patients with reactive BM were enrolled to assess hematogones. The patients were selected from 4 different hospitals in Baghdad. For each patient, peripheral blood and BMA from left over samples were taken to perform full blood count by automated device, peripheral blood film, BMA morphology, cytochemical stain using Periodic acid-Schiff (PAS) and Sudan black B (SBB) stains, the results of PAS stain were recorded as a percentage of such cells showing any PAS-positivity then they were classified into 3 groups, corresponding to less than 1% PAS positive cells, 1-10%, and over 10 %, respectively for correlation purposes [10].

For every patient at least 0.3 ml of K2 EDTA anti-coagulated bone marrow sample was collected for flow cytometry and tested within 48 hours. All flow cytometric analysis in this study was done by Cyflow® cube 6 flow cytometer from Partec Company in a private laboratory. Flow cytometry analysis was performed as in the fallowing: in cases of B-ALL, leukemic blasts were gated in the CD45 versus side scatter (CD45/SSC) histogram, and the expression of CD34, CD123 on this population was then assessed. For reactive BM (hematogones):the population was identified as hematogones by gating on CD45/very low SSC events [11-14] and then hematogones were subdivided 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, then the expression of CD123on these group were assessed. For post chemotherapy patients, two gates on blast region of CD45/ SSC plot were used and the expression of CD34, CD123 was then assessed. Clusters of at least 10-20 events must be captured and interpreted [15].

Statistical analysis

All statistical operations were done by SPSS version 18 programs. The measurement and tests were: mean and slandered deviation; chi

square (X^2) test for qualitative data, student t test for independent data. An association or difference was considered significant if the probability value (*P* value) was ≤ 0.05.

Result

Patients were divided into 2 groups: Adults (≥ 15 years old) comprised 10 patients and children (< 15 years) comprised 20 patients. The mean age of pediatrics B-ALL group was 4.75±3 years (mean ± SD); ranging (2 months – 12 years) at diagnosis, with male: female ratio of 1.5:1 and the peak incidence was found in the age group ≥ 5 years. The mean age of B-ALL adults' group was 35.9±11.76 years, ranging (18-50 years) with male: female ratio of 1.5:1.

According to FAB classification; L2 was the most common subtype (80% and 60%) for adult and children respectively followed by L1. Regarding PAS studies on diagnostic blast cells, 9 (30%) patients had no PAS-positive material, while 21 patients (70%) had more than 1% PAS positivity, with 16 cases (53.33%) showing a strong reaction in over 10% of cells. All B-ALL cases included in this study were SBB negative (Table 1).

Table 1. Distribution of B-ALL patients according to WBC count, Hemoglobin concentration, platelet count, blast % in BM, FAB subtype and PAS %

Feature		Children (N = 20) N (%)	Adults (N = 10) N (%)	P value
WBC count x10^9/L	< 50 ≥ 50	15 (75) 5 (25)	7 (70) 3 (30)	0.548
Hemoglobin (g/l)	< 80 80-100 > 100	12 (60) 1 (5) 7 (35)	4 (40) 2 (20) 4 (40)	0.360
Platelet x10^9/L	< 50 50-100 > 100	9 (45) 10 (50) 1 (5)	6 (60) 3 (30) 1 (10)	0.47
Blasts % in BM	< 90 ≥90	4 (20) 16 (80)	3 (30) 7 (70)	0.542
Blasts % in blood	Present absent	18 (90) 2 (10)	10 (100) 0 (0)	0.301
FAB	L1 L2	8 (40) 12 (60)	2 (20) 8 (80)	0.419
PAS %	< 1 1-10 > 10	2 (10) 3 (15) 15 (75)	7 (70) 2 (20) 1 (10)	0.001

PAS = Periodic acid-Schiff, < 1% PAS positive cells (negative PAS), 1-10% (positive PAS), and over 10% (strongly positive PAS), FAB = French–American–British classification, WBC = white blood cell count.

Immunphenotyping of newly diagnosed B-ALL: in 28 (93.4%) cases, leukemic blasts expressed both CD34 and CD123, Conversely, in 2 (6.6%) cases, neither antigen was expressed. Thus, the expression of CD34 and CD123 was found to be concordant (either either positive or both negative) in all B-ALL cases (Fig. 1).

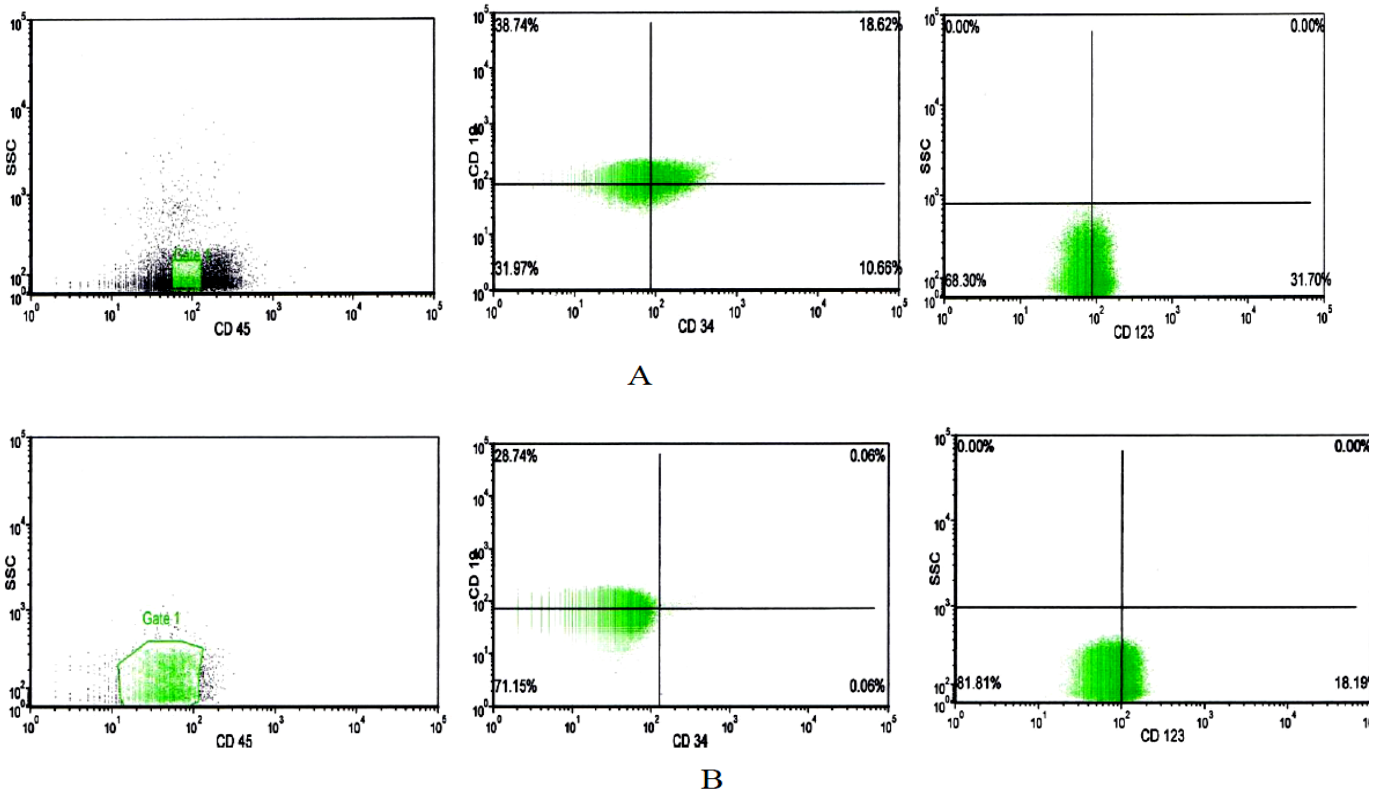


Fig. 1. Expression patterns of CD34 and CD123 on (B-ALL) blasts cases of B-ALL with pattern in which blasts express CD34 and CD123 antigens (A) blasts lack both antigens (B).

Regarding patients with hematogones (HGs), Age of patients ranged between 1.5 to 30 years, the median age was 2.75 year (hematogones occur in larger numbers in most normal marrow specimens of infants and young children but they are found in low numbers in most normal adult marrow specimens analyzed by flow cytometry (FC)) [(9)] with males: females ratio was 1:2.3. Hematogones cells by morphology show a spectrum of size and the exhibited features varied from mature lymphocytes to lymphoblast; HGs did not exhibit the PAS block- positive characteristic pattern of B- ALL blast and they were negative for Sudan black B (SBB). All cases of reactive BM specimens studied, hematogones were identified by flow cytometry, the less mature hematogones that had dim CD45, CD34+, not expressed CD123, whereas the more mature hematogones (moderate CD45+) lack CD34 but expressed CD123.Thus the expression of CD123 was found to be discordant in relation to CD34 in both groups of hematogones (Fig. 2).

The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and hematogones respectively had been studied in 20 B-ALL patients (4-6) weeks post chemotherapy (7 females and 13 males). In three (15%) of twenty cases, residual leukemic blasts were detected by FC. Five (25%) cases had residual leukemic blasts and late hematogones detected by FC (Fig. 3). In all of the above cases, the expression pattern of these two antigens (in blasts) remained constant after chemotherapy. For the remaining 12 cases (60%) HGs were detected only by FC.

In order to study relationship between expressions of both CD123, CD34 after induction therapy and the gender, age, (PAS% and WBC at initial diagnosis) and BM morphology after chemotherapy; the patients were divided after induction course of chemotherapy into two groups depending on response to induction therapy assessed by FC: First group included patients not achieved remission who had residual blast (concordant expression of CD34

and CD123), they were eight (40%). Second group included patients in remission that had only hematogones (discordant expression of CD34 and CD123) and they were twelve (60%) cases. A statistically significant difference in response to induction therapy ($P < 0.05$) was recorded with age of patients, initial PAS% and BM morphology after chemotherapy while the gender of patients or initial white blood cell count had no significant influence on response to chemotherapy (Table 2).

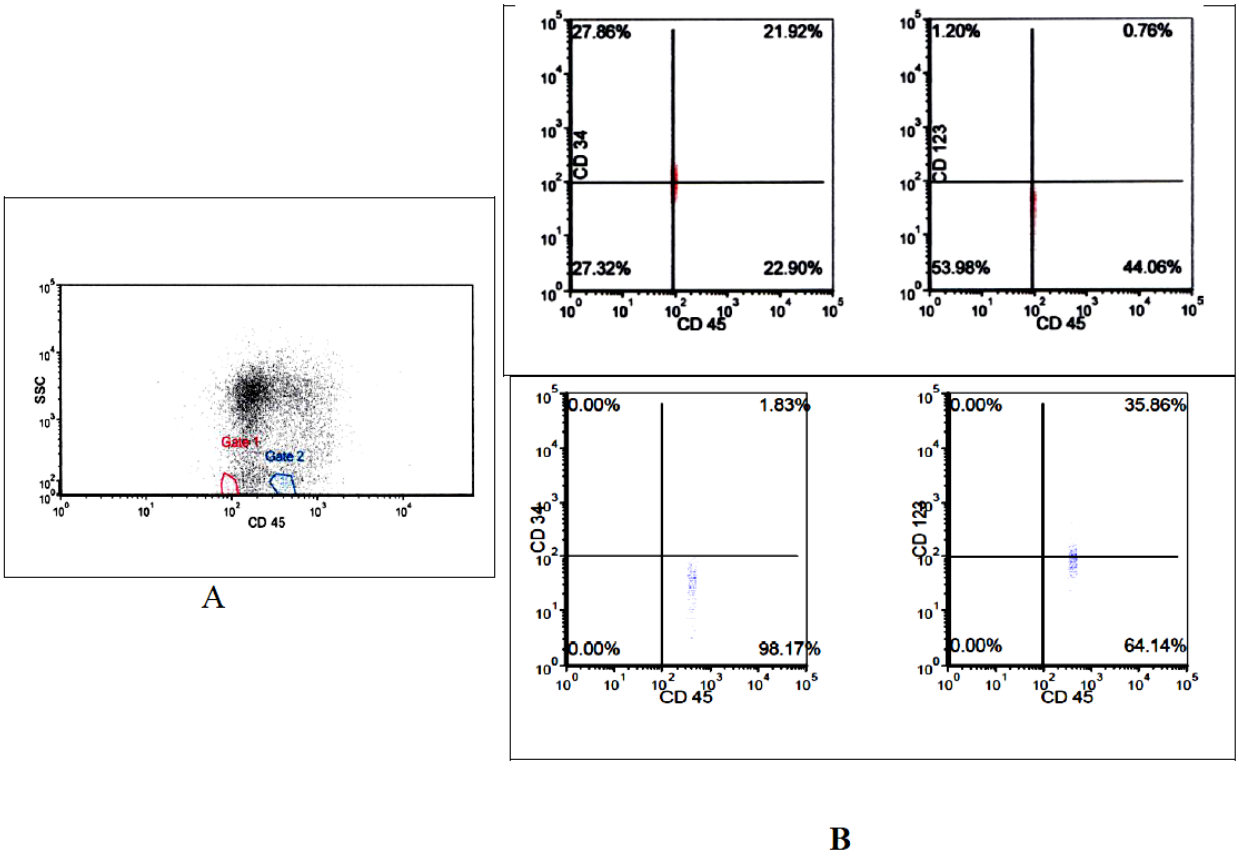


Fig. 2. Expression patterns of CD34 and CD123 on immature and mature hematogones. *A*, hematogones;the less mature hematogones with dim CD45 expression are gated in (gate 1 or red dots). The more mature hematogones with higher CD45 expression are gated in (gate 2 or blue dots). *B*, The upper left histogram shows the immature hematogones express CD34. The upper right plot shows that the same population is negative for CD123 expression and the lower left histogram show that the mature hematogones is negative for CD34 expression but express CD123 (lower right).

Discussion

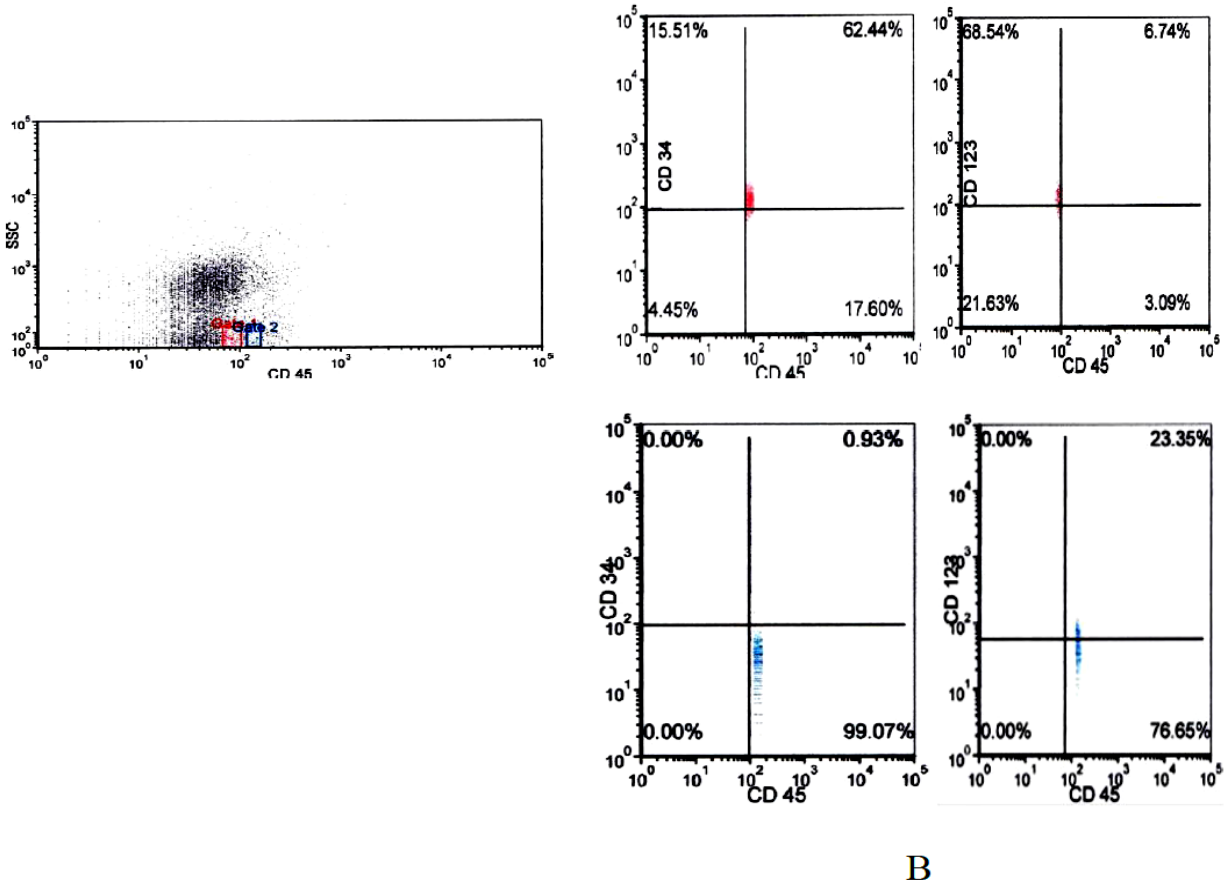
In this study, the mean age of pediatric B-ALL cases was 4.75 year with peak age of incidence was less than five years, these finding were comparable with other national study [16] and other literature in international populations [17]. The mean age of adult B-ALL patients was (35.9 year) lower than other Iraqi study [18] which was 46 years. This difference may be due to small sample size in current study.

In the present study, 25 % of pediatrics patients had WBC counts at diagnosis higher than $50x10^9$/L which confer poor prognosis; these findings go in line with many other studies [8,19] which showed that the WBC count was higher than 50 $x10^9$/L in 26%, 29% of pediatric ALL patients, respectively.

In current study, the WBC count among adult patients in 70 % of them was less than 50 $x10^9$/L while in 30 % of cases were over 50 $x10^9$/L These

figures were near the result recorded by Mancini *et al* [20].

Periodic Acid Schiff (PAS) studies on diagnostic blast cells showed that (30%) patients had no PAS-positive material, whereas 70% of patients had positive PAS material, these finding go in line with other observation [21] which showed that the PAS positive was in 66.66% of B-ALL cases.



B

Fig. 3. CD34 and CD123 expression patterns in treated B-acute lymphoblastic leukemia (B-ALL). A, Representative histograms shows residual blasts in (gate 1or red dots) and hematogones in (gate 2 or blue dots). B, residual blasts (red dots) that express CD34 and CD123 in the upper parts. The hematogones (blue dots) in the lower parts show discordant patterns of CD34 and CD123 expression.

Immunphenotyping of newly diagnosed B-ALL

It had been found that in 93.4% of cases leukemic blasts expressed both CD34 and CD123. Conversely, in 6.6% cases, neither antigen was expressed. These results confirmed the results obtained by Hassaneien and coworkers [9] who found that in 80% of B-ALL cases CD123 expression was associated with CD34 expression; whereas 11% expressed neither. Hematogones (HGs) in current study showed that small percentage of cells was indistinguishable morphologically from the lymphoblasts of B-ALL, this morphologic feature go in line with other investigator [4,7].

In some patients, the increase in HGs can be pronounced, resulting in confusion with ALL lymphoblasts. This is particularly true following treatment of ALL because hematogones are often expanded in regenerating marrow and can potentially be mistaken for residual disease [22,23].

Table 2. Responses to induction therapy and association with age, gender of patients, (WBC and PAS % at presentation) and BM morphology after chemotherapy

Feature		Patients in remissions (N = 8) N (%)	Adults (N = 12) N (%)	P value
WBC count x10^9/L	< 50 ≥ 50	15 (75) 5 (25)	7 (70) 3 (30)	0.548
Hemoglobin (g/l)	< 80 80-100 > 100	12 (60) 1 (5) 7 (35)	4 (40) 2 (20) 4 (40)	0.360
Platelet x10^9/L	< 50 50-100 > 100	9 (45) 10 (50) 1 (5)	6 (60) 3 (30) 1 (10)	0.47
Blasts % in BM	< 90 ≥ 90	4 (20) 16 (80)	3 (30) 7 (70)	0.542
Blasts % in blood	Present absent	18 (90) 2 (10)	10 (100) 0 (0)	0.301
FAB	L1 L2	8 (40) 12 (60)	2 (20) 8 (80)	0.419
PAS %	< 1 1-10 > 10	2 (10) 3 (15) 15 (75)	7 (70) 2 (20) 1 (10)	0.001
Age	≤ 15 > 15	11 1	4 4	0.035
Gender	Male Female	5 3	8 4	0.199
WBC count at initial presentation	< 50×10^9/L ≥ 50×10^9/L	4 4	10 2	0.06
BM morphology after chemotherapy	Blast < 5% blast ≥ 5%	5 3	12 0	0.021
PAS % at presentation	< 1% 1-10%	5 2	2 1	0.023

PAS = Periodic acid-Schiff, <1% PAS positive cells (negative PAS), 1-10% (positive PAS), and over 10% (strongly positive PAS), WBC = white blood cell count, Blast < 5% = complete morphological remission, blast ≥ 5% = incomplete morphological remission.

Because of these potential diagnostic difficulties, awareness should be taken for distinction of leukemic lymphoblasts in B acute lymphoblastic leukemia (B-ALL) from their non-neoplastic counterparts in bone marrow (hematogones).

One important distinguishing characteristic of HGs in our patients was In bone marrow smears, HGs did not exhibit the block-positive of PAS pattern characteristic of ALL, these finding accepted by other investigator [14,24] and negative for SBB, Other investigators have shown that HGs are nonreactive with SBB [23].

This study had used four-color flow cytometry to define precisely the patterns of normal antigen expression on a series of normal bone marrows using CD34, CD123 and it has been found that the less mature hematogones (dimCD45+) that expressed CD34 lack CD123 expression, whereas the more mature hematogones (moderate CD45+) lacked CD34 but always express CD123.

These findings were in agreement with other studies [7] who found that CD123 was negative in normal lymphoid progenitors (CD34+CD33-CD19 + CD10+) and with Djokic *et al* [6] study who found that the early B-cell precursors were CD123 negative while intermediate precursors and mature B cells showed weak CD123 expression.

Other investigators have also reported methods for discriminating between normal B-cell precursors and neoplastic lymphoblasts. Farahat and associates [25], using quantitative double-labeling flowcytometry, found B-lineage ALL lymphoblasts to express fewer TdT and CD19 and more CD10 molecules than did hematogones. While McKenna *et al* [22] found that the concurrent expression of earliest and last antigen e.g. CD34 and CD20 in B-ALL but discordant expression in HGs.

The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and HGs respectively had been studied in twenty of B-ALL patients 4-6 weeks post chemotherapy. In 8/20 (40%) cases in whom remission was not achieved, residual blasts were presented by FC while 12/20 (60%) of remitted cases, had only hematogones assayed by FC. This finding is comparable to that recorded by Delbuono *et al* [26]; who found that about half of the patients had detectable residual daises (RD) in the BM (44% on day 14 and 39% on day 28) and was lower than that recorded by Hassaneien *et al* [9], who reported that 62% of cases had residual blast.

The current study tested the relationship of response to induction therapy with respect to other prognostic factors and found that there was significant correlation between response to induction therapy and BM morphology after chemotherapy, PAS % at initial diagnosis and the age of patients but no significant correlation presented with gender of patient and initial WBC. Delbuono *et al* [26]; agreed in his study with fact that there was correlation between RD and BM morphology but he found that RD was not significantly associated to gender, age and white blood cell count .

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Author Contribution

Research proposal was done by Musa, collection of samples, patients' interview, sample analysis, and patients follow up were done by Shallan and the final printout of article was done by both authors.

Conflict of Interest

The authors declare no conflict of interest.

Finding

Self funding.

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