Original Article

Prognostic value of myeloid antigens expression in childhood acute lymphoblastic leukemia

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Abstract:

BACKGROUND: Leukemic blasts in acute lymphoblastic leukemia (ALL) may have immunological features of both lymphoid and myeloid lineages known as aberrant myeloid antigens expression in ALL, which are explained as abnormal genetic program of leukemic cells that lead to lineage infidelity. In this study, CD13, CD14, and CD33 (which are the most frequent myeloid antigens associated with aberrant antigens expression in ALL) were investigated.

OBJECTIVES: To evaluate the occurrence of aberrant myeloid antigens expression in childhood ALL and its effects on complete remission and other parameters.

MATERIALS AND METHODS: This study was conducted on 31 pediatric patients with newly diagnosed *de novo* ALL (27 B-ALL, 4 T-ALL) in Children Welfare Teaching Hospital/Medical City of Baghdad; diagnosis of ALL based on morphology and cytochemistry, CD13, CD14, CD33 were investigated as myeloid antigens using four-color flow cytometry.

RESULTS: Five cases of ALL (16.13%) out of 31 cases were confirmed to have aberrant myeloid antigens expression (CD13 was expressed in all five cases, CD33 was expressed in three cases, and CD14 was not expressed in any of these five cases). Complete remission was achieved in 90.30% (28 patients) and all cases with aberrant myeloid antigens expression achieved complete remission; however, despite this, there was no significant difference in complete remission between myeloid-positive (MY+) and myeloid-negative (MY−) cases, $P > 0.05$. Regarding other parameters, there were significant statistical differences in lactate dehydrogenase (LDH), hemoglobin value, and bone marrow (B.M.) blasts percent at diagnosis between MY+ and MY− cases, $P < 0.05$; however, there was no significant differences in leukocytes count, platelets count, peripheral blood blast percent at diagnosis, age, and gender, $P > 0.05$.

CONCLUSIONS: The most frequent aberrant myeloid antigens expression in childhood ALL is CD13 and less frequently is CD33 while CD14 showed no expression; myeloid antigens expression in ALL may have better prognosis as they have lower B.M. blast percent and LDH value.

Keywords:
Aberrant, acute lymphoblastic leukemia, myeloid antigens

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by the accumulation of lymphoblasts in peripheral blood (P.B.), bone marrow (B.M.), and occasionally central nervous system. Sometimes, myeloid-associated antigens expressed in ALL are known as aberrant myeloid antigens expression in ALL and explained as abnormal genetic program of leukemic cells that lead to lineage infidelity.[1-3]

Myeloid-positive (MY+) ALL is a diverse group of disorder, so it is important to understand their biologic features which may lead to the development of the therapeutic plan of these patients; however, the clinical and prognostic significance of these aberrant expressions remain

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controversial. Previously, some reports established that MY+ ALL indicates to poor prognosis while others indicate to good prognosis and others established no independent prognostic significance.[1,2,4-6]

The incidence of aberrant myeloid antigens expression in children accounts for about 5%–49%; the conflicting reports about the occurrence of aberrant antigens expression in childhood ALL may be due to the variation in the reagent used against CD surface antigens, variation in the cutoff level, fresh or frozen sample which are used for analysis, and differences in phenotypic characterization of leukemic cells in adult and children.[7-10]

In this study, the frequency of conventional and aberrant antigens will be investigated in childhood ALL patients and diagnosed and treated at one institution, to detect if the presence of these aberrant antigens has a prognostic significance.[11]

Flow cytometry (FCM) is an important part of ALL diagnosis; previously, detection of aberrant myeloid antigens expression in lymphoblastic leukemia had been difficult and complicated using nonspecific monoclonal antibodies, but now, using a panel of antibodies specific for each lymphoid lineage and myeloid lineage allows feasible detection of both lineage and stage of differentiation of leukemic cells.[12-15]

This panel includes at least one highly sensitive marker (CD19 for B-ALL, CD7 for T-ALL, CD13 and CD33 for myeloid cells) and also includes antibodies to a highly specific marker (CD79, CD22 for B-ALL, CD3 for T-ALL, MPO for myeloid cells); this provides a firm diagnosis in about 99% of the cases.[16,17]

CD13 is a member of type II transmembrane glycoprotein also known as aminopeptidase N, usually expressed in all steps of myeloblasts maturation to granulocytes, so it is considered as a pan-myeloid marker;[18] CD13 expresses in the surface of blast cells of AML in a ratio 80% and has a higher cytoplasmic expression.[19] Hence, it has a high sensitivity when it uses technique that allows cytoplasmic antigens detection.[20,21]

CD33 is type I transmembrane protein presents in colony forming unit of myeloblasts, promyelocytes, monocytes, and erythrocytes, so it is considered as a pan-AML marker.[18,22] CD33 expressed in blast cells of AML in about 80%.[19,23]

CD13 and CD33 are considered classical myeloid antigens and most frequently associated with these aberrant myeloid antigens expressions in ALL.[18,24]

CD14 is a transmembrane protein usually presents in monocytes, neutrophils, macrophages, and sometimes on B-cells and dendritic cells, involved in transduction of signals which are associated with oxidative burst, and tumor necrosis factor α synthesis also may expressed in patients with nonlymphocytic leukemia and in B-chronic lymphocytic leukemia.[25,26]

CD14 usually expressed alone without another myeloid antigens, so its expression represents an aberration more than activation of a certain program of differentiation.[12]

Materials and Methods

After taking consents from all patients, a prospective cross-sectional study was conducted on fresh samples obtained from 31 pediatric patients (age <15 years) with newly diagnosed ALL who were admitted from January 2016 to June 2016 to Hematology Department in Welfare Pediatric Hospital/Medical City.

The diagnosis of ALL was made on routinely stained P.B. and B.M. aspirate smears and evaluated according to French-American-British criteria and also on cytochemical stain of B.M. aspirate film (Sudan black B, periodic acid-Schiff stain).

Hepatic function, renal function, coagulation, and biochemical tests were performed for all patients in addition to the physical examination and imaging study; then, flow cytometric analysis was performed on P.B. samples, the sample was transferred in cool box within few hours after collection in ethylenediaminetetraacetic acid tube to be investigated for myeloid antigens expression (CD13, CD14, CD33) using four-color FCM, and suitable gate was detected depending on FSC/SSC gate.

The sample was considered to be positive when the percentage of positive blast cells was equal to or >20%.

Results

After flow cytometric analysis, five cases out of the total 31 cases of ALL expressed aberrant myeloid antigens expression, CD13 expressed in all these five cases, while CD33 expressed in three cases and CD14 not expressed in any case [Table 1 and Figure 1].

In this study, only pediatric age group was collected and the age distribution among this age group was divided into three categories as >1 year, between 1 and 9 years, and >10 years, the highest frequency of ALL cases was in age group 1–9 years, and the least frequency was in patient <1 year; according to the results of this study, there was no significant statistical differences in age between MY+ cases and myeloid-negative (MY−) cases.
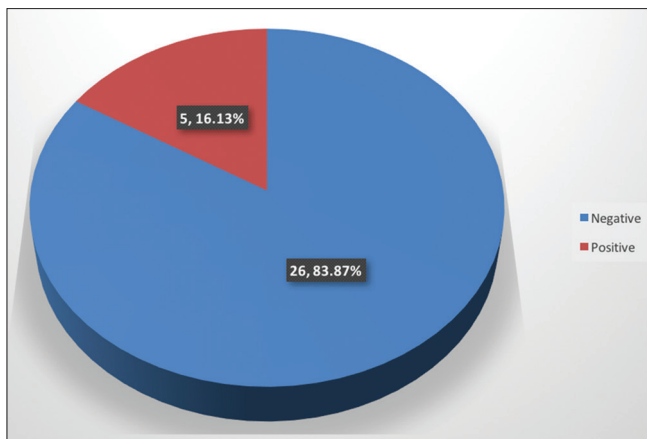
Figure 1: Percent of aberrant myeloid antigens in acute lymphoblastic leukemia

Figure 2: Flow cytometry histogram of one case of myeloid-positive acute lymphoblastic leukemia

According to the collected sample in our work, the gender distribution of ALL was nearly equal between male and female and M: F ratio = 1.08:1.

According to the results, there were significant statistical differences in hemoglobin (Hb) value, lactate dehydrogenase (LDH), and B.M. blast percent between MY+ ALL and MY− ALL, $P < 0.05$ (LDH, B.M. blast percent less in MY+ ALL and Hb value more in MY+ ALL at diagnosis); however, other hematological parameters such as white blood cell (WBC) count, platelet (PLs) count, P.B. blast percent, clinic reasoning (CR), and clinical presentation, there were no significant statistical differences between MY+ and MY− ALL cases [Tables 2-4 and Figure 2].

Discussion

Five cases out of 31 patients with conventional ALL expressed aberrant myeloid-associated antigens; this finding was compatible with that established by Pui *et al*. in which MY+ ALL cases account for about 16.4%[27] and less than that found by Kurec *et al*., Amirghofran *et al*., Tanyeli *et al*. in which MY+ ALL cases account for 49%, 30%, 48.4%, respectively.[4,11,28] The differences in these outcomes may be due to the differences in definition (in some reports, considered MY+ ALL as two or more myeloid-associated antigens positive, while in other reports, considered MY+ ALL as one or more myeloid-associated antigens positive) and also may be due to variation in number of monoclonal antibodies which were used in addition to ethical differences which may play a role in these variations.[1]

In the current work, there were no significant statistical differences in achieving complete remission between MY+ and MY− ALL cases despite all MY+ cases achieved CR and three out of 26 of MY− cases were not achieved CR and this finding was compatible with Wiersma *et al*.,
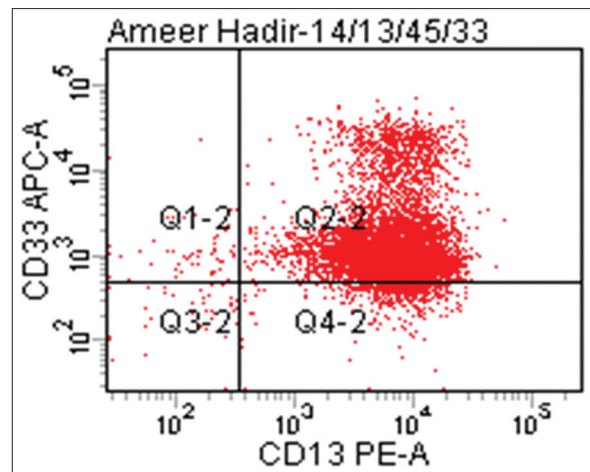
Table 1: Percent of each myeloid antigens expression in acute lymphoblastic leukemia

	Positive (%)	Negative (%)
CD13	5 (16.13)	26 (83.87)
CD14	0	31 (100.00)
CD33	3 (9.68)	28 (90.32)

Table 2: Correlation of myeloid antigens with hemoglobin value

Hb	Antigen	
	Negative	**Positive**
Mean	7.66	9.9
SE	0.44	0.98
Median	7.13	10.2
Percentile 25	6.06	8.7
Percentile 75	9.59	11.6
P	0.048	

Hb = Hemoglobin, SE = Standard error

Table 3: Correlation of myeloid antigens with bone marrow blasts percentage

Blast BM	Antigen	
	Negative	**Positive**
Mean	86.47	71
SE	2.73	5.42
Median	90.5	74
Percentile 25	83	72
Percentile 75	94	74
P	0.005	

SE = Standard error, BM = Bone marrow

Amirghofran *et al*. which also introduced that there were no significant statistical differences in CR between MY+ and MY− ALL cases.[11,12] This finding may be due to that there were no significant differences in most hematological parameters between MY+ and MY− cases or may be due to small sample size of all cases in general and MY+ ALL cases particularly, while in contrast to

Table 4: Correlation of myeloid antigens with lactate dehydrogenase value

LDH	Antigen	
	Negative	Positive
Mean	406.04	235.4
SE	66.16	10.96
Median	289.5	233
Percentile 25	253	215
Percentile 75	357	254
P		0.026

LDH = Lactate dehydrogenase, SE = Standard error

that findings of Bhushan B *et al*. which introduced that MY+ cases achieved CR more than MY− cases[2] and also discordant with Kurec *et al*. which reported that MY+ ALL achieved CR less than MY− cases.[4]

Uckun *et al*.'s results are generally consistent with these studies, in showing that myeloid antigen expression does not correlate with poor outcome for children with ALL and emphasize that their study provides new insight on the clinical significance of myeloid antigen expression in childhood ALL and shows that regardless of risk classification, ALL patients who are MY+ have treatment outcomes similar to those who are MY− ALL.[29]

Furthermore, the correlation between myeloid antigens expression and their effects on hematological parameters was evaluated in this study and found that there were no significant statistical differences in initial WBC and PLs count; these findings were compatible with that established by Bhushan B *et al*. and Kurec *et al*.[2,4] and discordant with that reported by Lopes *et al*., Uckun *et al*., which considered that MY+ ALL cases usually have initial WBC count less than MY− ALL and more initial PLs count.[18,29]

While in the current study, significant statistical differences were found in Hb value at diagnosis (Hb value usually more in MY+ cases than MY− cases) and LDH value at diagnosis (LDH value usually less at diagnosis in MY+ cases than MY− cases), this result was approachable with that established by Pui *et al*.[27] and conflicting with Tanyeli *et al*., Uckun *et al*., Wiersma *et al*. which instituted that there were no differences between MY+ and MY− ALL cases in these values[12,28,29] and also unfriendly with Amirghofran *et al*. which founded that CD33 usually associated with low Hb value.[11]

According to initial P.B. blasts and B.M. blasts percent, there were no significant statistical differences in P.B. blasts percent between MY+ and MY− cases, but there were significant statistical differences in initial B.M. blasts percent between MY+ and MY− ALL cases (B.M. blasts percent less in MY+ ALL than in MY− ALL

at diagnosis); this finding was compatible with that reported by Lopes *et al*.[18]

The differences about the prognosis of myeloid antigens expression in ALL between studies may be due to the differences in treatment protocol, definition criteria of myeloid antigens expression in ALL, and populations.[1]

Conclusions

1. CD13 is the most frequent aberrant myeloid antigens which was expressed in childhood ALL, less frequently CD33, while CD14 showed no expression
2. MY+ ALL may have better prognosis as they have lower value for B.M. blast percent and LDH
3. There was no difference in response to induction therapy between MY+ and MY− ALL.

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Conflicts of interest
There are no conflicts of interest.

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