Original Article

Access this article online

Quick Response Code:

Website: www.ijhonline.org

DOI: 10.4103/2072-8069.198119

Evaluation of CD96 and CD123 in CD34+ leukemic stem cells in acute myeloid leukemia patients and their relation to response to induction therapy

Haidar H. Al-Fatlawi, Raad Jaber Musa

Abstract:

BACKGROUND: Leukemic stem cells (LSCs) are thought to originate either from normal hematopoietic stem cells or from more differentiated progenitor cells. LSCs are capable of self-renewal, proliferation, and differentiation into malignant blasts.

OBJECTIVE: To evaluate the expression of the LSC markers CD96 and CD123 in de novo acute myeloid leukemia (AML) patients, and to explore the relationship between those markers and response to induction therapy and prognostic factors in AML.

MATERIALS AND METHODS: A cross-sectional study was conducted on 30 adults with newly diagnosed AML patients were prospectively tested for the expression of CD96 and CD123 using four-color flow cytometer at the time of diagnosis and re-evaluated at day 28 from the start of chemotherapy for the response to 3 + 7 induction therapy regimen.

RESULTS: Eight cases (26.7%) expressed CD96, and 12 cases (40%) expressed CD123; all the CD96 positive cases were also CD123 positive, however, four cases among the CD123 positive patients did not express CD96. CD96 and CD123 were expressed more on blast cells in the cases of M5 French–American–British subtype, whereas the least expression was in M3. Among the eight cases with CD96+ expression, only (37.5%) acquired CR, whereas cases without CD96 expression, (77.3%) acquired CR. Among the 12 cases with CD123+ expression, only (33.3%) acquired CR, while cases without CD123 expression, (88.9%) acquired CR.

CONCLUSION: The expressions of CD96 and CD123 were associated with a higher total white blood cell count and bone marrow blast cells at presentation, and a lower response rate to the induction therapy.

Key words:

Acute myeloid leukemia, CD123, CD34, CD96, flow cytometry

Acute myeloid leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is defined by the accumulation of leukemic blast cells, mainly in the marrow resulting in hematopoietic failure.[1] Iraq is among the world countries with both high incidence and low survival rate of AML.[2,3] Laboratory data suggest that AML originates from a rare population of cells, termed leukemic stem cells (LSCs), which are capable of self-renewal, proliferation, and differentiation into malignant blasts. Various surface markers have been assigned to LSCs such as CD96 and CD123, though there is universal agreement that LSCs exist within the CD34+ compartment of hemopoietic cells and the majority of LSCs are CD38−.[4]

LSCs might originate from malignant transformation of normal hematopoietic stem cells (HSCs), or alternatively, of progenitors in which the acquired mutations have re-installed a dysregulated self-renewal program. Most leukemia cells are initially sensitive to chemotherapy and radiotherapy, LSCs are resistant and are considered the basis for disease relapse after the initial response.[5] Similar to normal HSCs, LSCs are characterized by their ability to self-renew, unlimited repopulating

Department of Pathology and Forensic Medicine, College of Medicine, Alnahrain University, Baghdad, Iraq

Address for correspondence:
Dr. Haidar H. Al-Fatlawi, Department of Pathology and Forensic Medicine, College of Medicine, Alnahrain University, Baghdad, Iraq.
E-mail: dr.haider79@gmail.com

Submission: 15-08-2016
Accepted: 30-08-2016

How to cite this article: Al-Fatlawi HH, Musa RJ. Evaluation of CD96 and CD123 in CD34+ leukemic stem cells in acute myeloid leukemia patients and their relation to response to induction therapy. Iraqi J Hematol 2016;5:161-6.

potential and the production of a multitude of progeny cells with more differentiated characteristics.[6]

CD45 is a transmembrane tyrosine phosphatase ubiquitously expressed by white blood cells (WBCs). CD45 expression is high on lymphocytes and monocytes, whereas granulocytes, precursor B-cells, precursor granulocytic cells, and proerythroblasts are also CD45 positive, but at lower levels. In contrast, more mature erythroid cells are generally CD45 negative. Therefore, when combined with SSC characteristics, CD45 can help distinguish the major leukocyte (sub) populations in bone marrow (BM).[7] Most blasts have decreased CD45 expression (blast cells tend to be located in the CD45 weak positive region). However, the blast gate by CD45/SSC contains various cell types in addition to myeloid blasts. Therefore, specific markers of blast cells are required for definitive identification of blasts (usually CD34 and CD117).[8]

CD34 is a surface glycophosphoprotein. It is a greatly glycosylated Type I transmembrane protein that is a member of the sialomucin family of surface molecule.[9] CD34 has been generally used for the identification and isolation of HSCs and progenitors.[10] CD34 is considered as a poor prognostic factor in newly diagnosed AML.[11]

CD96; also known as T-cell activation increased late expression is a Type I membrane protein that belongs to immunoglobulin superfamily. CD96 was previously introduced as a novel cell surface marker on NK cells and an antigen for T-cell.[12] CD96 is not expressed in the majority of normal HSC population; however, it is intensely expressed in CD34+ CD38− AML cells. Furthermore, CD96+ LSCs have a high ability for engraftment in mice. Therefore, it may be probable to use this molecule for LSC-targeted antibody therapy.[13]

CD123 is also known as interleukin-3 receptor alpha (IL-3Rα). IL-3R is a heterodimeric cytokine receptor consist of the alpha and beta units.[14] CD123 or IL-3Rα interacts with the beta chain (CD131) of the IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor receptors to create a high-affinity IL-3 receptor. Dependent on the cell type, IL-3 will bind to the high-affinity IL-3 receptor heterodimer which stimulates cell proliferation, differentiation or cell survival.[15] In normal hematopoiesis, the binding of IL-3 to IL-3R stimulates the development and survival of multilineage colonies from normal BM, while in leukemia, IL-3 induce a stimulation effect on most human AML blasts that proliferate in response to IL-3.[16]

Materials and Methods

This prospective cross-sectional study was conducted on 30 adults *de novo* AML patients were admitted to the Hematology Department of Baghdad Teaching Hospital and Al-Imamain Al-Kadhimian Medical City from May 2015 to February 2016. This research was approved by the Ethical Committee at the College of MEDICINE, Al-Nahrain University. For each patient, a questionnaire form was done. A volume of 2.5 ml of peripheral blood (PB) and 0.5 ml of BM aspirate was collected in EDTA tube from each patient. The hematological parameters were obtained by automated hematology analyzer (CELL-DYN Ruby). PB and BM films were stained with Leishman, Sudan Black B and periodic acid–Schiff stains. After morphological diagnosis of AML, the specimen was transferred in a cool box (within 6 h) to a private laboratory to be investigated for the expression of surface marker antigens (CD96 and CD123) using four-color flow cytometer (Partec Cyflow®Cube 6, Germany) and interpretation of markers by FACS Express Software. The parameters of CD45/SSC (dim/low expression of CD45 and low SSC) which was used to locate immature cells (Gate 1) then CD34 positive blasts were selectively gated (Gate 2).[17,18] From this population of cells, the expression of CD96 and CD123 immuophenotypes are defined when at least 20% of the blast cells expressed those markers.[18] For remission induction, the patients were categorized into high and low-risk groups by physician according to their age and WBC count at presentation (risk stratification); hence, those patients with age ≥60 years and WBC count ≥50×10^9/L at presentation were considered as a high-risk group and given less intensive therapy; while those patients with age <60 years and WBC count <50×10^9/L at presentation were considered a low-risk group and received the induction therapy protocol of "3+ 7" (3 days of an anthracycline "doxorubicin 30 mg/m^2 or daunorubicin 45 mg/m^2" and 7 days of cytarabine 100 mg/m^2 continuous intravenous [IV]).[19] The acute promyelocytic leukemia patients received different regimen (All-Trans Retinoic Acid (ATRA) 45 mg/m^2 daily until complete remission (CR) plus doxorubicin 30 mg/m^2 IV or idarubicin 12 mg/m^2 (IV in 30 min) for 4 doses at alternative days (D 2, 4, 6, and 8 of ATRA treatment).[20]

For all patients with AML, PB, and BM aspirate samples were repeated at day 28 from the start of chemotherapy to assess the CR, for AML M3 patients BM repeated only after recovery of PB hematological parameters.

Patients were classified according to Cheson criteria of response into CR (<5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells, there should be no blasts with Auer rods or persistence of extramedullary disease, and have an absolute neutrophil count of >1×10^9/L and platelets of ≥100×10^9/L), resistant disease (persistent leukemia by blood and/or BM examination) and death during induction.[21]

The statistical analysis of this prospective study performed with the Statistical Package for Social Sciences 21.0 (Armonk, NY: IBM Corp.) and Microsoft Excel 2013. Categorical data formulated as count and percentage. Chi-square test describes the association of these data. Numerical data with normal distribution were described as mean and standard deviation (SD), independent sample *t*-test used for comparison between two groups. While for abnormal distribution, Mann–Whitney test used for comparison between two groups. The lower level of accepted statistical significant difference is bellow or equal to 0.05.[22]

Results

Thirty adult newly diagnosed AML patients were enrolled in the study; the mean age was 41.33 ± 16.58 years (mean ± SD); the range was 16–75 years. The highest incidence was reported at the age group 25–34 years while lowest at ≥65 years. AML was observed more in males than in females with male:female ratio of 1.3:1. The mean of total WBC count at

presentation was 40.3 ± 59.83 × 10^9/L (mean ± SD), ranged from (1.5–234 × 10^9/L) and median was 15.4 × 10^9/L. Patients were divided into three groups according to the counts of WBCs: Leukopenia (<4 × 10^9/L), normal (4–10 × 10^9/L), and leukocytosis (>10 × 10^9/L). Accordingly, seven patients (23.3%) were leukopenic, seven patients (23.3%) had normal WBC count, whereas 16 patients (53.4%) which represent the majority of cases had leukocytosis [Table 1].

The mean of hemoglobin (Hb) concentration was 8.49 ± 2.66 g/dl (mean ± SD), the range was 3.96–15.3 g/dl and median was 7.99 g/dl. Only two patients (6.7%) had a normal Hb concentration. The mean platelet count was 60.41 ± 38.82 × 10^9/L (mean ± SD) and the range was 6.6–146 × 10^9/L. The median was 57.6 × 10^9/L, all patients (100%) had low platelet counts [Table 1].

French–American–British (FAB) M2 formed the major portion of AML subtypes (30.00%), followed by M3 (23.33%) and M5 (16.67%) then M1 and M4 (13.33% for each) and M7 was (3.33%), M0 and M6 subtypes were not represented in the current study [Table 1].

CD96 was expressed in 8/30 patients (26.7%), for CD123 expression, 12/30 patients (40%) had positive expressions. All CD96 positive cases were also CD123 positive, however, four cases among the CD123 positive patients didn't express CD96 [Figure 1]. CD96 was expressed more in M5 subtype cases, while not expressed in M3 subtypes. CD123 was expressed more in M5 subtype cases, while expression was poor in M3 [Table 2].

Regarding hematological parameters, the total WBC count and BM blast cells percentage of AML patients with CD96 and CD123 expression were significantly higher than those without markers expression, while there was no significant correlation between PB blast cells percentage, Hb level and platelet count with the CD96 and CD123 expression [Tables 3a, b, 4a and b].

CR was achieved in 20/30 patients (66.67%) at the end of induction therapy. Among the eight cases with CD96+ expression, only three cases (37.5%) acquired CR and five cases (62.5%) have no remission. On the other hand, among the 22 cases without CD96 expression, 17 cases (77.3%) acquired CR and only five cases (22.7%) have no remission. Out of the 12 cases with CD123+ expression, only four cases (33.3%) acquired CR and eight cases (66.7%) have no remission. While among the 18 cases without CD123 expression, 16 cases (88.9%) acquired CR and only two cases (11.1%) have no remission, and these results appeared that there was a significant correlation between positive expressions of CD96 and CD123 with the nonresponsiveness to induction therapy [Table 5].

Table 1: Distribution of acute myeloid leukemia according to gender, hematological parameters and French-American-British subtype

Variable	*n* (%)	Range	Mean±SD	Median
Gender				
Male	17 (56.67)	N/A	N/A	N/A
Female	13 (43.33)			
WBC count (×10^9/L)				
<4	7 (23.3)	1.5-234	40.3±59.83	15.4
4-10	7 (23.3)			
>10	16 (53.4)			
Hb (g/dl)				
Low*	28 (93.3)	3.96-15.3	8.49±2.66	7.99
Normal	2 (6.7)			
Platelet count (×10^9/L)				
Low**	30 (100)	6.6-146	60.41±38.82	57.6
FAB subtypes				
M1	4 (13.33)	N/A	N/A	N/A
M2	9 (30.00)			
M3	7 (23.33)			
M4	4 (13.33)			
M5	5 (16.67)			
M7	1 (3.33)			

*Low Hb if <13 g/dl in men and 12 g/dl in women, **Low platelet if <150×10^9/L (males and females).[23] WBC = White blood cell, SD = Standard deviation, N/A = Not available, Hb = Hemoglobin, FAB = French-American-British

Discussion

The mean age of patients was 41.33 years, which was in agreement with other Iraqi studies.[24-26] The peak incidence of AML was found in the age group 25–34 years, while the least incidence was found in the age group ≥65 years, these findings were comparable with the result of study made by Mohammed in 2014.[26] In this study, the mean age of patients and the peak incidence were markedly lower than that observed in the developed countries where the incidence of AML increased with age, and the majority of cases were above 55 years of age.[27] This might be attributed to small sample size or other factors related to the biology of leukemia. The male to female ratio was 1.3:1, which was in accordance with that reported by the Iraqi ministry of health[2] and other Iraqi studies.[24-26]

The mean WBC count, Hb level, and platelet count were comparable to previous Iraqi studies. Leukocytosis (WBC count >10 × 10^9/L) was found in 53.33% of AML patients which was more than that reported by Dawood[28] and less than results reported by Al-Mohsen[24] and Mohammed,[26] Whereas leukopenia and normal leukocyte count at presentation were comparable to observed findings in the other Iraqi studies by Hussein survey in 2013[3] and Al-Husseiny in 2008.[29] This difference might be attributed to small sample size. The mean Hb concentration and platelet count were comparable to other studies by Al-Mohsen[24] and Mohammed.[26]

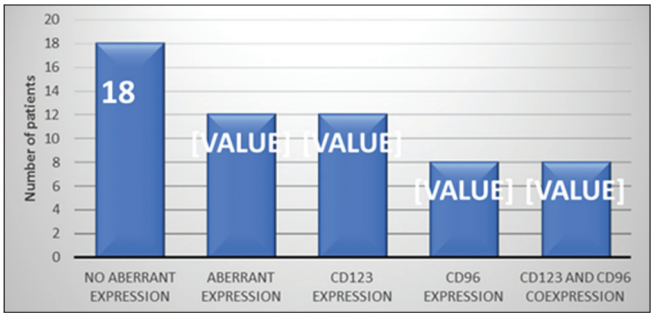

Figure 1: Frequency of cases expressing CD96 and CD123 in acute myeloid leukemia patients

Table 2: Distribution of CD96 and CD123 expressions in relation to acute myeloid leukemia subtypes

FAB subtype	CD96			CD123		
	Positive (%)	Negative (%)	Total	Positive (%)	Negative (%)	Total
M1	1 (25)	3 (75)	4	1 (25)	3 (75)	4
M2	2 (22.2)	7 (77.8)	9	3 (33.34)	6 (66.67)	9
M3	0	7 (100)	7	1 (14.28)	6 (85.72)	7
M4	1 (25)	3 (75)	4	2 (50)	2 (50)	4
M5	3 (60)	2 (40)	5	4 (80)	1 (20)	5
M7	1 (100)	0	1	1 (100)	0	1
Total	8	22	30	12	18	30

FAB = French-American-British

Table 3a: Correlation between CD96 expression with total white blood cell count

Parameter	CD96 positive (*n*=8)			CD96 negative (*n*=22)			*P*
	Mean	Median	Percentile (5-95)	Mean	Median	Percentile (5-95)	
Total WBC count (×10⁹/L)	83.05	47.55	3.61-234	24.75	9.6	1.58-100	0.003

WBC = White blood cell

Table 3b: Correlation between CD96 expression with hematological parameters

Parameter	CD96 positive (*n*=8)		CD96 negative (*n*=22)		*P*
	Mean±SD	SE	Mean±SD	SE	
Hb (g/dl)	8.23±2.27	0.80	8.58±2.69	0.57	0.741
Platelet count (×10⁹/L)	58.96±48.97	17.31	60.94±35.77	7.62	0.914
PB blast cells %	57.13±28.62	10.12	49.05±25.88	5.51	0.468
BM blast cells %	84.00±6.82	2.41	61.41±19.368	4.129	0.003

SD = Standard deviation, SE = Standard error, BM = Bone marrow, PB = Peripheral blood, Hb = Hemoglobin

Table 4a: Correlation between CD123 expression with total white blood cell count level

Parameter	CD123 positive (*n*=12)			CD123 negative (*n*=18)			*P*
	Mean	Median	Percentile (5-95)	Mean	Median	Percentile (5-95)	
Total WBC count (×10⁹/L)	62.34	30.65	1.63-234	25.6	7.6	1.5-160	0.002

WBC = White blood cell

Table 4b: Correlation between CD123 expression with hematological parameters

Parameter	CD123 positive (*n*=12)		CD123 negative (*n*=18)		*P*
	Mean±SD	SE	Mean±SD	SE	
Hb (g/dl)	8.19±2.66	0.77	8.69±2.53	0.60	0.607
Platelet count (×10⁹/L)	57.01±43.53	12.56	62.68±36.47	8.59	0.702
PB blast cells %	53.67±25.70	7.42	49.56±27.43	6.46	0.683
BM blast cells %	80.42±8.27	2.38	58.78±20.39	4.80	0.001

SD = Standard deviation, SE = Standard error, BM = Bone marrow, PB = Peripheral blood, Hb = Hemoglobin

Table 5: Correlation between CD96 and CD123 expression with complete remission achievement

Marker	Remission		Total	*P*
	Complete, *n* (%)	Not, *n* (%)		
CD96+	3 (37.5)	5 (62.5)	8	0.041
CD96–	17 (77.3)	5 (22.7)	22	
CD123+	4 (33.3)	8 (66.7)	12	0.002
CD123–	16 (88.9)	2 (11.1)	18	

Regarding FAB subtypes, FAB M2 was the most frequent (30%) followed by M3 (23.33%). These results were comparable to other Iraqi studies carried out by Al-Hemedawee in 2014 who reported that M2 was the most frequent followed by M5[25] and Alwan *et al.*, in 2009 showed that M2 was the most common subtype followed by M1.[30] However, other local previous studies done by Hussein,[3] Al-Mohsen,[24] Mohammed[26] and Al-Husseiny[29] found that FAB M3 was the most common subtype. The lower number of M3 cases in this study might be attributed to differences in sample sizes or due to environmental factors related to seasonal variation.[31]

The percentage of CD96+ expression cases was comparable to that found by Gramatzki *et al.*,[12] Zhao *et al.*,[17] and Du *et al.*,[32] but lower than that done by Hosen *et al.*[13] The total WBC count of AML patients with CD96 expression was significantly higher than those without this expression and this result was comparable to result documented by Zhao *et al.*, 2013.[17] The present work did not reveal any significant differences in CD96+ expression with respect to the PB blasts percentage. However, CD96 expression was significantly

associated with BM blasts percentage. To the best of our knowledge, no previous studies addressed the correlation of the PB blasts and BM blasts percentage with CD96 expression, interestingly in 2015, a study done on patients with myelodysplastic syndrome by Zhang *et al*., revealed that the expression levels of CD96 and CD123 were significantly correlated with the proportion of the BM blasts.[33] Regarding CD96 expression distribution among FAB subgroups, it showed that the high percentage of CD96+ observed in M5 subtype, while CD96 is not expressed in M3 subtype and these results were in agreement with Zhao *et al*.,[17] however, previous study done by Gramatzki *et al*., showed that CD96 expression is rare in M5 subtype.[12] Among the eight cases with CD96+ expression, only three cases (37.5%) acquired CR, while among the 22 cases without CD96 expression, 17 cases (77.3%) acquired CR. Current results were consistent with that of Zhao *et al*., 2013[17] and Du *et al*., 2015[32] who found that CD96+ expression was associated with a lower rate of CR in AML patients.

The percentage of CD123+ cases was in agreement with the studies done by Zhao *et al*., 2013[17] and Farweez *et al*., 2015,[18] on the other hand, this study result was higher than that reported by Zhu *et al*., 2012[34] and lower than that reported by previous an Iraqi study by Al-Mohsen in 2013[24] and Ge *et al*., 2014.[35]

The total WBC count of AML patients with CD123+ expression was significantly higher than those without this expression. Similar results were documented by several previous studies.[16,18,35,36] The present work did not reveal any significant differences in CD123+ expressions with respect to the PB blasts percentage, whereas it was significantly associated with BM blasts percentage. The same results had been documented by Farweez *et al*.,[18] and Khorshed *et al*.[36] Regarding CD123 expression distribution among FAB subgroups, it showed that highest percentage of CD123+ observed in M5 subtype, while the least expression was in M3 and these results were in agreement with Zhao *et al*.[17] Among the 12 cases with CD123 expression, only four cases (33.3%) acquired CR, while among the 18 cases without CD123 expression, 16 cases (88.9%) acquired CR and these results were similar to that found by Zhao *et al*., 2013,[17] Farweez *et al*., 2015[18] and Ge *et al*., 2014[35] who found that CD123 expression was associated with a reduced response to induction chemotherapy.

Conclusion

The Current study present that the expressions of CD96 and CD123 were associated with a higher total WBC count and BM blast cells at presentation, and a lower response rate to the induction therapy; thus CD96 and CD123 are considered as unfavorable markers gives evidence that these markers have clinical association

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Liesveld JL, Lichtman MA. Acute myelogenous leukemia. In: Lichtman MA, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT, editors. Williams Hematology. 8th ed. China: The McGraw-Hill Companies, Inc.; 2010. p. 1277-330.
2. Iraqi Cancer Registry: Leukemia Statistic of the Year 2009. Minstry of Health/Iraqi Cancer Board; 2011.
3. Hussein SH. Survey of Adult *de novo* Acute Myeloid Cases Reported in the Medical City in Baghdad Through the Years (2005-2011). A Thesis Submitted to the Department of Pathology and Forensic Medicine in partial fulfillment of Master of Science Degree in Hematology: College of Medicine-Baghdad University; 2013.
4. Jordan CT. The leukemic stem cell. Best Pract Res Clin Haematol 2007;20:13-8.
5. Buss EC, Ho AD. Leukemia stem cells. Int J Cancer 2011;129:2328-36.
6. Ho AD, Wagner W. Bone marrow niche and leukemia. Ernst Schering Found Symp Proc 2006;5:125-39.
7. van Lochem EG, van der Velden VH, Wind HK, te Marvelde JG, Westerdaal NA, van Dongen JJ. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: Reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 2004;60:1-13.
8. Kussick SJ, Wood BL. Using 4-color flow cytometry to identify abnormal myeloid populations. Arch Pathol Lab Med 2003;127:1140-7.
9. Krause DS, Fackler MJ, Civin CI, May WS. CD34: Structure, biology, and clinical utility. Blood 1996;87:1-13.
10. Nielsen JS, McNagny KM. Novel functions of the CD34 family. J Cell Sci 2008;121(Pt 22):3683-92.
11. Junghanss C, Waak M, Knopp A, Kleine HD, Kundt G, Leithäuser M, *et al.* Multivariate analyses of prognostic factors in acute myeloid leukemia: Relevance of cytogenetic abnormalities and CD34 expression. Neoplasma 2005;52:402-10.
12. Gramatzki M, Ludwig WD, Burger R, Moos P, Rohwer P, Grünert C, *et al.* Antibodies TC-12 ("unique") and TH-111 (CD96) characterize T-cell acute lymphoblastic leukemia and a subgroup of acute myeloid leukemia. Exp Hematol 1998;26:1209-14.
13. Hosen N, Park CY, Tatsumi N, Oji Y, Sugiyama H, Gramatzki M, *et al.* CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A 2007;104:11008-13.
14. Testa U, Pelosi E, Frankel A. CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. Biomark Res 2014;2:4.
15. Sun Q, Woodcock JM, Rapoport A, Stomski FC, Korpelainen EI, Bagley CJ, *et al.* Monoclonal antibody 7G3 recognizes the N-terminal domain of the human interleukin-3 (IL-3) receptor alpha-chain and functions as a specific IL-3 receptor antagonist. Blood 1996;87:83-92.
16. Testa U, Riccioni R, Militi S, Coccia E, Stellacci E, Samoggia P, *et al.* Elevated expression of IL-3Ralpha in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. Blood 2002;100:2980-8.
17. Zhao M, Zhu H, Sajin R, Xiao X, Deng Q. Clinical significance of leukemia stem cells immunophenotype expression in patients with acute leukemia. Life Sci J 2013;10:2544-8.
18. Farweez BA, Hashem AE, Elsalakawy WA. Leukemia stem cell markers: CD 123 and CD25 are poor prognostic markers in adult acute myeloid leukemia patients. Int J Adv Res 2015;3:136-47.
19. Dombert H, editor. Optimal Acute Myeloid Leukaemia Therapy in 2012 Haematology Education: The Education Program for the Annual Congress of the European Haematology Association; 2012.
20. Kotiah SD. Acute Promyelocytic Leukemia Treatment Protocols:

WebMD LLC; 2014. Available from: http://www.emedicine.medscape.com/article/2005126-overview. [Last updated on 2015 Dec 30].

21. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, *et al.* Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010;115:453-74.
22. Elliott AC, Woodward WA. Statistical Analysis Quick Reference Guidebook: With SPSS Examples. California: Sage Publication; 2007.
23. Bates I, Lewis SM. Reference ranges and normal values. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. UK: Elsevier Limited; 2011. p. 11-22.
24. Al-Mohsen FS. Identification of Leukemic Stem Cells in Acute Myeloid Leukemia by Flow Cytometry and its Correlation with Response to Induction Therapy. A Thesis Submitted to the Department of Pathology and Forensic Medicine in Partial Fulfillment of Master of Science Degree in Hematology: College of Medicine -Al Nahrain University; 2013.
25. Al-Hemedawee NS. Evaluation of the Aberrant Expression of CD56 and CD2 in Newly Diagnosed Adult Acute Myeloid Leukaemia. A Thesis Submitted to the Scientific Council of Pathology in Partial Fulfillment of Requirements for the Degree of Fellowship of the Iraqi Board for Medical Specialization in Pathology/Hematology; 2014.
26. Mohammed RS. Expression of CD13 and CD33 in *de novo* Adult Acute Myeloid Leukaemia and Their Clinical Relevance A Thesis Submitted to the Scientific Council of Pathology in Partial Fulfillment for the Requirements for the Degree of Fellowship of the Iraqi Committee for Medical Specializations in Pathology/Haematology; 2014.
27. Legrand O, Perrot JY, Baudard M, Cordier A, Lautier R, Simonin G, *et al.* The immunophenotype of 177 adults with acute myeloid leukemia: Proposal of a prognostic score. Blood 2000;96:870-7.
28. Dawood SD. Assessment of IL-6 serum level in patients with acute myeloid leukemia. Iraqi J Cancer Med Genet 2011;4:22-8.
29. AL-Husseiny AH. Acute myeloid leikemia in adolescent and adult Iraqi patients. Diala J 2008;29:353-62.
30. Alwan AF, Zedan ZJ, Salman OS. Acute myeloid leukemia: Clinical features and follow-up of 115 Iraqi patients admitted to Baghdad Teaching Hospital. Tikrit Med J 2009;15:1-8.
31. Mele A, Stazi MA, Pulsoni A, Visani G, Monarca B, Castelli G, *et al.* Epidemiology of acute promyelocytic leukemia. Haematologica 1995;80:405-8.
32. DU W, Hu Y, Lu C, Li J, Liu W, He Y, *et al.* Cluster of differentiation 96 as a leukemia stem cell-specific marker and a factor for prognosis evaluation in leukemia. Mol Clin Oncol 2015;3:833-8.
33. Zhang W, Shao Z, Fu R, Wang H, Li L, Liu H. Expressions of CD96 and CD123 in bone marrow cells of patients with myelodysplastic syndromes. Clin Lab 2015;61:1429-34.
34. Zhu HB, Zhao MF, Li YM. Relationship between the expression of leukemia stem cell immunophenotype and the treatment efficacy and prognosis. Zhonghua Xue Ye Xue Za Zhi 2012;33:58-60.
35. Ge F, Li B, Gao X, Ren Y, Liu H, Su B, *et al.* Immuno-phenotypes and prognosis of acute leukemia in elderly patients. Int J Clin Exp Med 2014;7:3714-8.
36. Khorshed A, Elsharkawy N, Elrefaey FM, Elgamal B. Interleukin-3 receptor alpha chain as a unique marker for leukemic stem cells in acute myeloid leukemia. Life Sci J 2011;8:68-7.