ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Leukemia Stem cell Markers: CD 123 and CD25 are poor prognostic markers in adult Acute Myeloid Leukemia Patients

Botheina Ahmed Thabet Farweez [1], Azza El-Sayed Hashem[1], Walaa Ali Elsalakawy[2]

1. Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

2.Department of Internal Medicine, Clinical Hematology and Bone Marrow Transplant Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Manuscript Info

Manuscript History:

Received: 14 October 2015
Final Accepted: 22 November 2015
Published Online: December 2015

Key words:

Leukemia stem cell markers, Prognosis, Acute myeloid leukemia.

Corresponding Author

Botheina Ahmed Thabet Farweez

Abstract

Background: Acute myeloid leukemias (AML) still remain a challenge for hematologists. Though an impressive number of prognostic factors have been identified in AML, it still ranks one of the highest cancer related deaths. Several studies have indicated their origin from a rare population of leukemic cells, known as leukemic stem cells, which initiate the disease and contribute to frequent relapses. Leucocyte interleukin-3 receptor α (CD123) and leucocyte interleukin-2 receptor α (CD25) are regarded as markers of leukemia stem cells.

Aim: The aim of this study was to investigate CD123 and CD25 expression in newly diagnosed patients with AML by flow cytometry and correlate their expression with disease prognostic parameters and patients' outcome at day 28 of therapy.

Patients and Methods: This study was conducted on 30 newly diagnosed patients with AML admitted to Ain Shams University Hospitals; Egypt. They were subjected to full medical history and clinical examination, complete blood count with examination of peripheral blood and bone marrow (BM) smears, routine immunophenotyping of BM or whole peripheral blood and cytogenetic studies. The expression of CD123 and CD25 was performed using the following panel where gated blast cells were stained for CD45, CD38, CD34, CD123 and CD25.

Results: In the current study, CD123 was expressed in 13/30(43.3%) and CD25 was expressed in 4/30(13.3%). CD123 expression positively correlated with higher total leucocytic count and BM blast percentage and CD25 expression. Both CD123 and CD25 expression had a significantly poor effect on outcome even in the good prognostic cytogenetic subgroups.

Conclusion: Results of our study clearly demonstrate the poor prognostic significance of CD123 and CD25 expression in AML patients. This may represent additional prognostic tool in risk stratified management of AML patients.

INTRODUCTION

Acute myeloid leukemia (AML) originates from a special proportion of leukemia stem cells (LSC) which possess self-renewal capacity and are responsible for the continued growth and proliferation of the bulk of leukemia cells. It

is believed that LSC are also the root cause for the treatment failure and relapse of AML because LSC are often resistant to chemotherapy. [1]

Leukemia stem cells (LSC) and hematopoietic stem cells (HSC) share similar $CD34^{+}CD38^{-}$ surface immunophenotype, the search of cell surface markers unique to LSC or at least differentially expressed has attracted intensive enthusiasm in hematology and oncology field. Such markers will provide excellent therapeutic windows for specifically targeting LSC, while sparing normal HSC and are expected to be much tolerable for AML patients. [2]

CD123 is the alpha chain of interleukin-3 receptor (IL3-R) and CD25 is the alpha chain of the interleukin-2 receptor (IL-2R), they have been shown to be highly expressed on LSCs. [1]

The objective of the present study was to investigate CD25 and CD123 expression by flow cytometry technique at diagnosis in Egyptian AML patients and their relationship with disease prognostic parameters and patients' outcome at day 28 of therapy, aiming to apply them in routine clinical practice.

SUBJECTS AND METHODS

This study included 30 newly diagnosed adult AML patients admitted to and followed up at the Clinical Hematology Oncology Unit, Ain Shams University Hospitals; cairo; Egypt in the period from January 2013 to December 2014. Patients' diagnosis, management and follow up were performed according to WHO classification [3] and European Society for Medical Oncology (ESMO) Clinical Practice Guidelines for diagnosis, treatment and follow-up of AML in adult patients. [4] Patients' characteristics were evaluated at diagnosis by history, physical examination, complete blood count using Coulter LH 750 analyzer, examination of Leishman-stained peripheral blood (PB) and bone marrow (BM) aspiration smears. Routine diagnostic immunophenotyping of the bone marrow (BM) aspirate was performed on EPICS XL coulter Flow cytometer using a panel of monoclonal antibodies including: B cell markers: CD10, CD19, CD20, T cell markers: CD2, CD3, CD5, CD7. Myeloid markers: CD13, CD33, CD15, CD17 and monocytic marker: CD14. Common progenitor markers: CD34, HLA-DR, CD38. Cytoplasmic markers: MPO, CD79a and CD3. Samples were considered positive for a certain marker when ≥ 20% of cells were expressing it, except for CD34 where its expression by 10% of cells was sufficient to confer positivity. [5] Conventional karyotyping and Fluorescence In Situ Hybridization (FISH) in selected cases were performed. Patients were followed up at the day 28 from the beginning of the induction therapy.

Induction therapy:All patients were subjected to induction chemotherapy by standard dose cytarabine plus anthracyclin (7+3) : Ara-c 100 mg/m2 for 7 days. Daunorubicin60 mg/m2 for 3 days.

Informed consent was obtained from all participant individuals. The study was conducted in accordance with the stipulations of the local ethical and scientific committees of Ain Shams University; Egypt and the procedures respected the ethical standards in Helsinki declaration of 1964.

Flow cytometric assessment of CD123 and CD25:

For each sample analyzed, in addition to the test tube, one control tube was required for isotopic matched controls. For a blood sample, optimal staining was obtained using a number of leukocytes between 5 and 10 x 10^3 cells/ µL. If the leucocyte concentration was greater than 10 x 10^3 cells/ µL, it was diluted with phosphate buffer saline (PBS) (pH 7.2+/- 0.2).

Sample staining:

1. 50µL of prepared sample containing at least 5×10^3 cells were added to each sample tube.
2. 5µL of each PE-conjugated anti-CD123 monoclonal antibody and FITC conjugated anti-CD25 monoclonal antibody, PC7 conjugated anti-CD45, ECD conjugated anti-CD38 and PC5 conjugated anti-CD34 (R&D systems, UK) were added to each sample tube.
3. Tubes were incubated for 15 to 20 minutes at room temperature and protected from light. 1-2ml of ammonium chloride-based erythrocyte lysing solution were added to every tube and incubated for 5-10 minutes. Tubes were vortexed then washed with PBS (pH 7.2 +/- 0.2). Cells were suspended in 0.5 ml PBS and analyzed using Navios flowcytometer (Coulter electronics, USA).

Data interpretation:

Analysis was performed using Navios software where 10,000 events were analyzed per case. A five-color flow cytometric assay protocol was constructed in which CD45/SSC gating was used to locate immature cells then CD38

negative blasts were selectively gated. The expression of CD123 (cutoff 20%) [6], CD25 (cutoff 20%) [cutoff for positivity for CD25 was variable between studies; Terwijn et al., 2009[7] and Cerny et al., 2013[8] suggested using a cutoff ≥10% based on the maximal expression of CD25 on CD34 positive myeloid blasts in normal marrow, while, Gönen et al., 2012[9] used the cutoff level of 20% as it has been known as the standard, and we consider this as the best cut-off level to exclude false-positive results] and CD34 (cutoff 10%) were assessed in terms of percentage of expressing cells (Figure 1).

STATISTICAL ANALYSIS:

Statistical analysis performed using SPSS V17. Quantitative data were represented as mean and standard deviation for parametric data and as median and range in non parametric data. Qualitative data were represented as number and percentage.

Comparisons of qualitative variables were conducted between groups using the Chi-square and comparisons of quantitative variables were conducted between groups using the Mann Whitney for non parametric data and student T test for parametric data. While, comparisons between more than two groups with parametric distribution was done by using One Way Analysis of Variance (ANOVA) and Kruskal-Wallis for non parametric distributions.

In addition, correlations between quantitative variables within groups were performed using the Pearson correlation coefficient. $p<0.05$ and <0.001 were set as statistically significant and highly significant respectively.

RESULTS:

- **Baseline characteristics:**

The studied patients included 13 (43.3%) males and 17 (56.6%) females, with M: F ratio of 1:1.3. Their age ranged from 20 to75 years, with a mean value of 44.00 years ± 16.88. They were classified according to FAB Grouping, but none of the patients were diagnosed as M6 or M7. (see table 1)

Conventional cytogenetic analysis was performed where 3 (10%) out of 30 patients showed failed mitosis and could not perform cytogenetic analysis. The remaining 27 patients were divided into: 14 patients out of 27 (51.9%) had normal karyotype by conventional analysis and the other 13 patients performed FISH analysis. Patients were further classified as favorable, unfavorable and intermediate prognostic groups according to cytogenetic analysis where t (8;21) (q22;q22), inv16 (p13;q22), and t(15;17) (q22;q21) were considered as favorable prognosis, while normal karyotype was considered intermediate prognosis and 11q23 rearrangement was considered unfavorable prognosis.[10] Expression of CD34, CD123 and CD25 was performed on the gated blast cells which were CD38 negative. Studied laboratory data are shown in Table (1).

- **Comparative analysis:**

Relation between CD123 and different parameters:

There was statistical significant association between CD123 expression and each of total leucocytic count (TLC) and BM blast cells percentage (%); higher median value for TLC and BM blast cells % was found in $CD123^+$ patients (P-value = 0.002 and 0.013 respectively). Also, CD123 expression and FAB subtypes showed a statistical significant relation (P-value = 0.039), where higher percentage (38.46%) of $CD123^+$ expression was found among M4 FAB subtype. Moreover, there was a statistical significant association between CD25 expression and CD123 expression (P-value = 0.006) where the 4 patients positive for CD25 were CD123 positive. However, there was no statistical significant relation between CD123 expression and either demographic, clinical data or other studied laboratory parameters (Table 2).

Relation between CD123 CD34 co-expression and different parameters:

There was no statistical significant association as regards CD123 CD34 co-expression and any of the studied parameters.

Relation between CD25 and different parameters:

There was no statistical significant association as regards CD25 expression and any of the studied parameters except for a statistical significant relation with CD123 expression (Table 3).

- **Outcome of AML patients:**

On assessing patients' outcome at day 28; 12 patients (40%) achieved hematological remission while, 10 patients (33.3%) didn't achieve hematological remission and 8 patients (26.7%) died shortly after their diagnosis.

Relation of outcome and studied parameters:

The relationship between the outcome and FAB subtypes showed a statistical significant relation (p-value = 0.048). Higher percentage of patients who didn't achieve hematological remission at day 28 were found among M4 FAB subtype (40.0%) and higher percentage of patients who died were found among M5 FAB subtype (37.5%) subtype

while, higher percentage of patients who achieved hematological remission at day 28 were found among M2 FAB subtype (50.0%)(Table 4).

There was statistical significant relation between the outcome and different markers expression where all $CD25^+$ cases died shortly after diagnosis and they were $CD123^+$ also. While, $CD123^+$ cases; 8 out of 13 didn't achieve hematological remission and 5 out of 13 died shortly after diagnosis (Figure 2). Moreover, It was of notice that 8 patients out of the 10 patients who didn't achieve hematological remission showed CD123 co-expression and 6 patients of them showed CD123 CD34 co-expression (Table 4); higher median value for CD123 expression and CD123 CD25 co-expression were found among patients who died shortly after diagnosis (p-value = 0.012 and 0.048 respectively) (Table 5).

There was statistical significant relation between different cytogenetic aberrations and patients' outcome at day 28 (p-value = 0.033). Patients with normal karyotype were 14 patients (51.8% of total cytogenetically studied patients) they represented 75% of patients who died, 50% of patients who didn't achieve hematological remission and 36.4% of patients who achieved hematological remission but it was noticed that 27.3% of patients who achieved hematological remission presented with t(8;21) or t(15;17) (good prognostic cytogenetic group) and although inv 16 is one of the good prognostic cytogenetic aberrations, 3 patients out of 4 with inv 16 did not achieve hematological remission (Table 4) and those patients were found to have co-expression of CD123 CD25.

- **Correlation Analysis:**

CD34 had statistically significant negative correlation with BM blasts (r: -0.405, p-value: 0.026), CD25 had statistically significant positive correlation with CD123 (r = 0.399, p-value = 0.029) and CD123 had statistically significant positive correlation with TLC and BM blasts (r = 0.365, 0.553 and p-value = 0.048, 0.002 respectively).

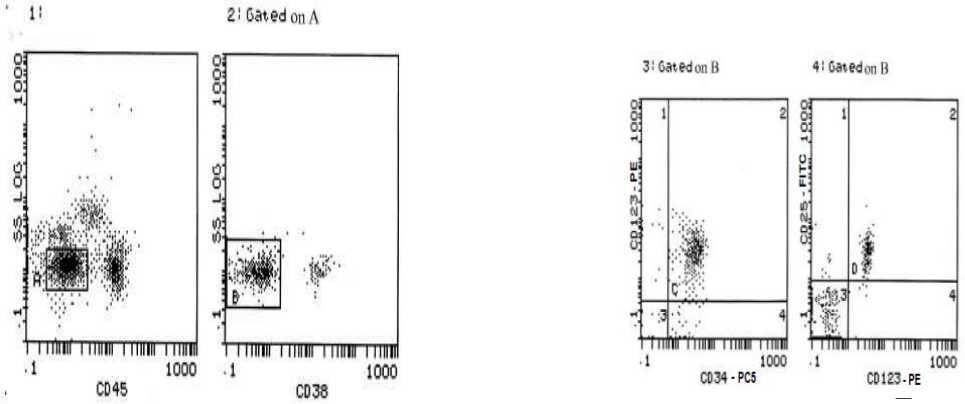

Figure (1): Representative flow cytometry scatters diagrams for CD123/CD25/CD34 on $CD38^-$ blast cells in one of the studied AML cases

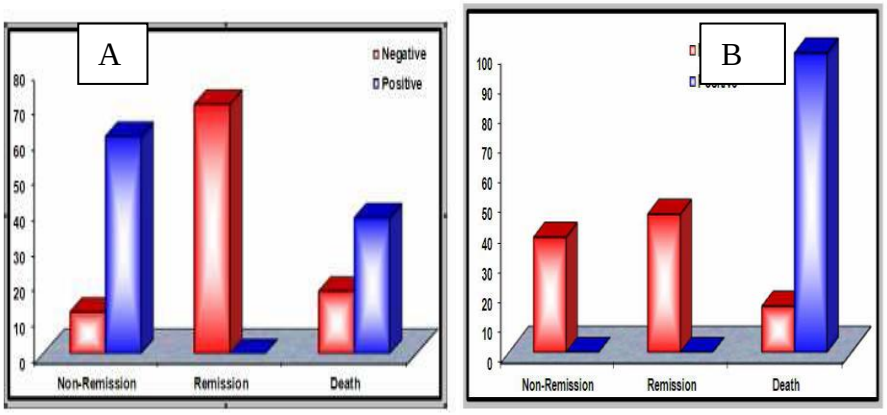

Figure (2): A .Relation between CD123 expression and the outcome of studied AML patients. B. Relation between CD25 expression and the outcome of studied AML patients

DISSCUSION:

AML is a clinically and biologically heterogeneous disease for which prognostic factors have become increasingly important for the choice of the appropriate therapy. New prognostic tools based on biological analyses which are accurate and easily available in clinical settings are needed. [11]

Leukemia stem cells are reported to play a crucial role in the development and progression of many hematological malignancies, including AML. [3] CD123 and CD25 have been reported from the LSC markers; such markers will provide excellent therapeutic windows for specifically targeting LSC, while sparing normal HSC and are expected to be much tolerable for AML patients. [2] The current study was conducted to evaluate the clinical value of investigating CD25 and CD123 expression at initial diagnosis as prognostic factors for AML via investigating their correlations with other well-established prognostic factors of biologic relevance and patients' outcome at day 28 of induction therapy.

In agreement with other previous studies [12, 13, 14 and 15] this study showed that CD123 was expressed on the blast cells in 43.3% of patients. There is some variation in the reported percentage of positivity for CD123 between these studies ranging from 40 to >90%, which could be explained by the different methodologies used to assess CD123 expression (flow cytometry versus immunohistochemistry), variation in number of studied patients and variation of the cells on which CD123 expression was tested. However, it could be concluded that CD123 is very frequently expressed on AML blasts and enforces previous reports about its role in AML development or pathology.

On exploring the relation between the expression of CD123 and studied laboratory data, CD123 was associated with higher TLC and higher percentage of BM blasts. This might suggest that the expression of CD123 probably offers a proliferation advantage to malignant cells and explain the association with poor prognosis as they are prognostic factors in themselves, similar results were documented by several previous studies. [13, 14, 15 and 16]

The relation of CD123 to proliferation advantage have been emphasized by the study of Testa et al., 2002 [13] who sorted leukemic cells according to strong or low expression of CD123 and concluded that cells expressing CD123 displayed higher growth activity but lower differentiation ability, and exhibited increased resistance to apoptosis triggered by growth factor (IL3) deprivation.

The distribution of CD123 expression among the FAB subgroups showed the highest percentages of CD123 positivity observed in M4 FAB subtype and none of the M1 FAB subtype was positive. Ehninger et al., 2014 [17] found a high percentage for CD123 positivity of 80-90% in M4, M4eos, M0, M1, M5 and 100% of M3 and M6 leukemia but they showed lower percentage for CD123 positivity in M2 FAB. However several studies did not find a significant difference in CD123 expression and FAB grouping. [13, 15 and 16] This might suggest that its prognostic impact is not confined to a specific group even in the subgroups of AML with favorable outcome.

A significant correlation between CD123 expression and CD25 expression was found where all $CD25^+$ patients (4 patients) were $CD123^+$. Similarly, Gönen et al., 2012[9] performed Gene Expression Profiling (GEP) analysis in $CD25^+$ intermediate risk patients and observed increased expression of CD123 in this subgroup.

Upon grouping patients according to their cytogenetic prognostic groups, no significant difference in CD123 expression and the three cytogenetic prognostic groups was found but it was of note that 6 out of 11 (54.5%) positive for CD123 were in the intermediate prognostic groups. In agreement, Ehninger et al., 2014 [17] reported the same finding and suggested that generally patients might profit to the same extent from targeted therapies against CD123 and CD33 as only around 4% of AMLs were negative for both marker.

Previous work done by Riccioni et al., 2011[18], Rollins-Raval et al., 2013[19] and Ehninger et al., 2014[17] who studied the relation of CD123 and molecular aberrations; they had shown higher CD123 expression in fms like tyrosine kinase-internal tandem duplication ($FLT3\text{-}ITD^+$) AMLs compared with FLT3 wild-type allele (FLT3-WT) thus this enforces the poor prognostic impact of CD123 expression.

CD25 was positive in 13.3% of patients in the present study and showed a significant relation with CD123 expression but not with any other studied clinical and laboratory parameters. Likewise, Gönen et al., 2012[9] and Cerny et al., 2013[8] reported nearly same percentage of positivity. Meanwhile, Ikegawa et al., 2014[20] and Gönen et al 2012[9] reported that CD25 expression was associated with higher TLC in addition to higher percentage of peripheral blood blast cells. [9]

There was no significant relation between CD25 expression and the three cytogenetic prognostic groups but it was noticed that 50% of CD25+ cases were found in the cytogenetically intermediate-risk AML (mostly with normal karyotype). Also, Terwijn et al., 2009[7], Cerny et al., 2011[8] and Gönen et al., 2012[9] reported similar results. Additionally, they demonstrated that $CD25^+$ patients with intermediate risk cytogenetics have a greater likelihood of harboring unfavorable risk mutations compared with CD25 cytogenetically intermediate risk patients. Thus, this highlights the great importance of CD25 expression especially in the intermediate cytogenetic risk group patients.

Previous work by Gönen et al., 2012 [9] had shown a high frequency of $FLT3\text{-}ITD^+$ cases in $CD25^+$ AML (76% of cases) and documented that CD25 $FLT3\text{-}ITD^+$ patients fared equally well as CD25 FLT3-WT patients suggesting that lack of CD25 expression outweighs the well-known adverse prognostic effect of the FLT3-ITD mutation. They

recommended that CD25 status should be considered an important covariate in the selection of patients for therapeutic trials with FLT3-kinase inhibitors

As regards the patients' outcome at day 28 after induction, poor outcome (death shortly after diagnosis or non remission state) showed a highly significant relation with CD123 expression, significant relation with CD25 expression, significant relation with CD123 CD25 co-expression and CD123 CD34 co-expression. This is in line with Testa et al., 2002 [13] who reported that patients with CD123 over expression had a lower complete remission and survival duration. Also, Vergez et al., 2011[11] reported that the number of $CD34^+/CD123^+/CD$ cells was predictive of AML patients outcome and a proportion of CD34+/CD123+/CD38 cells greater than 15% in AML patients and an unfavorable karyotype was associated with a lack of complete remission; furthermore, the presence of more than 1% of $CD34^+/CD123^+/CD38$ cells had a negative impact on disease-free survival and overall survival Similarly, Gönen et al., 2012[9] reported that CD25 expression was associated with a reduced response to induction chemotherapy. In addition, several independent reports using diverse patient cohorts established a significant positive correlation between the percentage of the CD25 positive leukemic blast population and poor overall survival or relapse free survival. [7, 8]

In conclusion, we suggest that CD25 and CD123 can be incorporated as additional biomarkers to improve prognostication in AML and these patients should be considered for tailored immunotherapies targeting CD123 and CD25 which are likely to enhance treatment efficacy in AML patients. Moreover, the study of the stability of these markers during the course of the disease and its applicability as a marker for MRD should be performed.

Conflict of interest: the authors declare no conflict of interest

References:

[1] Zhou J and Chng W. Identification and targeting leukemia stem cells: The path to the cure for acute myeloid leukemia; World J Stem Cells, 2014; 6(4): 473-84.

[2] Witte K, Ahlers J, Schafer I, Andre M, Kerst G, Scheel-Walter H et al. High proportion of leukemic stem cells at diagnosis is correlated with unfavorable prognosis in childhood acute myeloid leukemia. Pediatr Hematol Oncol, 2011; 28: 91-9.

[3] Swerdlow S, Campo E, Harris N, Jaffe E, Pileri S, Thiele J et al (Eds): World Health Organization (WHO) classification of tumors: pathology and genetics of haematopoietic and lymphatic tissues, 4th edition, 2008. Lyon, France: IARC Press. Chapter 6:110-23.

[4] Fey M. and Buske C. on behalf of the ESMO Guidelines Working Group. Acute myeloblastic leukaemias in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol., 2013; 24 Suppl 6:vi138-43.

[5] Matutes E, Morillo R and Morillo A. Immunophenotypic analysis of acute myeloid leukemia. In: Dacie and Lewis Practical Hematology (eds. Lewis S. Bain B. and Bates I), 11th edition, 2012. Elsevier, Churchill, Livingstone, China, Chapter 16: 362-4.

[6] Jordan C, Upchurch D, Szilvassy S, Guzman M, Howard D, Pettigrew A et al. The interleukin-3 receptor alpha is a unique marker for human acute myelogenous leukemis stem cells. Leukemia, 2000, 14(10):1777-84.

[7] Terwijn M, Feller N, van Rhenen A, Kelder A, Westra G, Zweegman S et al. Interleukin-2 receptor alpha-chain (CD25)expression on leukemic blasts is predictive for outcome and level of residual disease in AML. Eur J Cancer, 2009; 45(9):1692–9.

[8] Cerny J, Yu H, Ramanathan M, Raffel G, Walsh W, Fortier N et al. Expression of CD25 independently predicts early treatment failure of acute myeloid leukaemia (AML). Br J Haematol, 2013; 160(2):262-6.

[9] Gönen M, Sun Z, Figueroa M, Patel J, Abdel-Waheb O, Racevskis J et al. CD25 expression status improves prognostic risk classification in AML independent of established biomarkers: ECOG phase 3 trial, E1900. Blood, 2012; 120(11):2297–306.

[10] Betz B and Hess J. Acute myeloid leukemia diagnosis in the 21st century. Arch. Pathol. Lab. Med. 2010; 134(10):1427-33.

[11] Vergez F, Green A, Tamburini J, Sarry J, Gaillard B, Cornillet-Lefebvre P et al. High levels of CD34+CD38low/-CD123+ blasts are predictive of an adverse outcome in acute myeloid leukemia: a Groupe Ouest-Est des Leucemies Aigues et Maladies du Sang (GOELAMS) study. Haematologica, 2011; 96 (12):1792-8.

[12] Munoz L, Nomdedeu J, Lopez O, Cornier M, Bellido M, Aventin A et al. Interleukin-3 receptor alpha chain (CD123) is widely expressed in hematologic malignancies. Haematologica, 2001; 86(12):1261–9.

[13] Testa U, Riccioni R, Coccia E, Stellacci E, Samoggia P, Latagliata R et al. Elevated expression of IL-3R alpha in acute myelogenous leukemia is associated with enhanced bast proliferation, increased cellularity and poor prognosis. Blood, 2002; 100(8):2980–8.

[14] Riccioni R, Rossini A, Calabro L, Diverio D, Pasquini L, Lococo F et al. Immunophenotypic features of acute myeloid leukemias overexpressing the interleukin 3 receptor alpha chain. Leuk Lymphoma, 2004; 45(8):1511-7.
[15] Testa U., Riccioni R, Diverio D, Rossin A, Lo Coco F and Peschle C. Interleukin-3 receptor in acute leukemia, Leukemia, 2004; 18, 219–26.
[16] Khorshed A, Elsharkawy N, Elrefaey F, Mansour O and Elgamal B. Interleukin-3 Receptor Alpha Chain as a Unique Marker for Leukemic Stem Cells in Acute Myeloid Leukemia. Life Science Journal, 2011; 8(4): 68-74.
[17] Ehninger A, Kramer M, Rollig C, Thiede C, Bornha M, von Bonin M et al on behalf of the Study Alliance Leukemia. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia; Blood Cancer Journal, 2014; 4: e218-28
[18] Riccioni R, Pelosi E, Riti V, Castelli G, Lo-Coco F and Testa U. Immunophenotypic features of acute myeloid leukaemia patients exhibiting high FLT3 expression not associated with mutations. Br J Haematol, 2011; 153: 33-42.
[19] Rollins-Raval M, Pillai R, Mitsuhashi-Warita T, Metha R, Boyadzisn M, Djokic M et al. CD123 immunohistochemical expression in acute myeloid leukemia is associated with underlying FLT3-ITD and NPM1 mutations. Appl Immunohistochem Mol Morphol, 2013; 21(3):212–7.
[20] Ikegawa S, Doki N, Yamamoto K, Shingai N, Takahashi Y, Aoki J et al. Clinical impact of CD25 expression on outcomes of allogeneic hematopoietic stem cell transplant for cytogenetically intermediate-risk acute myeloid leukemia; Leuk Lymphoma,;56(6):1874-7.

Table (1): Laboratory data of studied patients

Qualitative Parameters	**N**		**%**
FAB subtype	M0	1	3.33
	M1	3	10
	M2	10	33.33
	M3	5	16.67
	M4	6	20
	M5	5	16.67
Cytogenetic studies (conventional karyotyping, FISH)	Normal	14	51.85
	t(8;21)	3	11.11
	inv 16	4	14.81
	t(15;17)	4	14.81
	11q23	2	7.41
Cytogentic Prognostic groups	Unfavorable	2	7.41
	Intermediate	14	51.85
	Favorable	11	40.74
CD34 expression	Negative	12	40
	Positive	18	60
CD123expression	Negative	17	56.67
	Positive	13	43.33
CD123 CD34 Coexpression	Negative	23	76.67
	Positive	7	23.33
CD25 expression	Negative	26	86.67
	Positive	4	13.33
CD123 CD25 Coexpression	Negative	26	86.67
	Positive	4	13.33
Quantitative Parameters	**Range**		**Median (IQR)**
CD34 (%)	1 - 98		32.50 (2.00 – 81.00)
CD123 (%)	0.46 - 48.90		16.85 (10.50 – 25.40)
CD123 CD34 coexpression (%)	0 - 47.9		3.31 (0.00 – 18.90)
CD25 (%)	0 - 25		0.08 (0.03 – 0.23)
CD123 CD25 coexpression (%)	0 – 25.01		0.13 (0.02 – 0.72)
TLC (x10^3/µl)	0.6-246		44.30 (6.30 – 72.00)
Hb (g/dl)	4.4-12.8		6.75 (5.60 – 8.00)
PLT (x10^3/µl)	5-371		35.50 (21.00 – 72.00)
PBB (%)	5-90		53.50 (20.00 – 79.00)
BM Blast cells(%)	20-95		77.50 (60.00 – 83.00)

TLC: Total leucocytic count; Hb: Hemoglobin; PBB: Peripheral blood blast cells percentage;
PLT: Platelet; BM: Bone marrow; N: number

Table (2): Relation between CD123 and different parameters:

Qualitative Parameters		CD 123 Expression				Test of significance	
		CD123 Negative		CD123Positive		Chi-Square	
		N	%	N	%	X^2	P-value
Sex	**Female**	9	52.94	8	61.54	0.222	0.638
	Male	8	47.06	5	38.46		
Hepatomegaly	**Negative**	13	76.47	9	69.23	0.196	0.658
	Positive	4	23.53	4	30.77		
Slpeenmegaly	**Negative**	13	76.47	9	69.23	0.196	0.658
	Positive	4	23.53	4	30.77		
Lympadenopathy	**Negative**	15	88.24	10	76.92	0.673	0.412
	Positive	2	11.76	3	23.08		
FAB subtype	**M0**	0	0.00	1	7.69	11.696	0.039*
	M1	3	17.65	0	0.00		
	M2	7	41.18	3	23.08		
	M3	4	23.53	1	7.69		
	M4	1	5.88	5	38.46		
	M5	2	11.76	3	23.08		
Cytogenetic studies (conventional karyotyping, FISH)	**Normal**	8	50.00	6	54.55	5.607	0.230
	t(8;21)	3	18.75	0	0.00		
	inv 16	1	6.25	3	27.27		
	t(15;17)	3	18.75	1	9.09		
	11q23	1	6.25	1	9.09		
Cytogentic prognostic groups	**Unfavorable**	1	6.25	1	9.09	0.184	0.912
	Intermediate	8	50.00	6	54.55		
	Favorable	7	43.75	4	36.36		
CD 25(%)	**Negative**	17	65.38	9	34.62	7.512	0.006*
	Positive	0	0.00	4	100.00		
CD34(%	**Negative**	6	50.00	6	50.00	2.340	0.126

Quantitative Parameters	CD 123 negative	CD123Positive	Mann-Whitney test	
	Median (IQR)	Median |(IQR)	Z	P-value
CD34 (%)	35 (1 – 81)	30 (2 – 78)	-0.148	0.883
CD123 CD34 coexpression (%)	2.48 (0 – 10.5)	23.9 (0 – 25)	-1.109	0.268
CD25 (%)	0.08 (0.03 – 0.2)	0.06 (0.04 – 20.2)	-0.547	0.585
CD123 CD25 coexpression (%)	0.11 (0 – 0.41)	0.21 (0.03 – 12.42)	-0.969	0.333
TLC ($x10^3/\mu l$)	6.6 (3 – 48.6)	70 (49.8 – 125)	-3.119	0.002*
Hb (g/dl)	6.8 (5.6 – 8)	6.7 (6 – 7.9)	-0.021	0.983
PLT ($x10^3/\mu l$)	36 (21 – 50)	33 (25 – 111)	-0.419	0.676
PBB (%)	40 (20 – 60)	60 (35 – 87)	-1.135	0.257
BM Blast cells (%)	69 (35 – 80)	80 (75 – 85)	-2.478	0.013*
Age (yrs) [mean± SD)	42.8 ± 14.5	44.8 ± 18.6	(t-test)-0.304	0.763

TLC: Total leucocytic count; Hb: Hemoglobin; PBB: Peripheral blood blast cells percentage;
PLT: Platelet; BM: Bone marrow; N: number; *: Significant p value

Table (3): Relation between CD25 and different parameters:

Qualitative Parameters		CD25 Expression				Test of significance	
		CD25 Negative		CD25 Positive		Chi-Square	
		N	%	N	%	X^2	P-value
Sex	Female	14	53.85	3	75.00	0.666	0.415
	Male	12	46.15	1	25.00		
Hepatomegaly	Negative	20	76.92	2	50.00	1.159	0.282
	Positive	6	23.08	2	50.00		
Slpeenmegaly	Negative	20	76.92	2	50.00	1.159	0.282
	Positive	6	23.08	2	50.00		
Lympadenopathy	Negative	22	84.62	3	75.00	0.210	0.647
	Positive	4	15.38	1	25.00		
Bleeding tendency	Negative	16	61.54	4	100.00	3.544	0.060
	Positive	10	38.46	0	0.00		
FAB Subtype	M0	1	3.85	0	0.00	6.420	0.268
	M1	3	11.54	0	0.00		
	M2	10	38.46	0	0.00		
	M3	4	15.38	1	25.00		
	M4	5	19.23	1	25.00		
	M5	3	11.54	2	50.00		
Cytogentic studies	Normal	12	52.17	2	50.00	3.898	0.420
	t(8;21)	3	13.04	0	0.00		
	inv 16	4	17.39	0	0.00		
	t(15;17)	3	13.04	1	25.00		
	11q23	1	4.35	1	25.00		
Cytogentic Prognostic groups	Unfavorable	1	4.35	1	25.00	1.694	0.429
	Intermediate	12	52.17	2	50.00		
	Favorable	10	43.48	1	25.00		
CD123 (%)	Negative	17	65.38	0	0.00	7.512	0.006*
	Positive	9	34.62	4	100.00		
CD34 (%)	Negative	9	34.62	3	75.00	2.340	0.126
	Positive	17	65.38	1	25.00		
$CD123^+CD34^+$ Coexpression (%)	Negative	20	76.92	3	75.00	0.007	0.933
	Positive	6	23.08	1	25.00		

Quantitative Parameters	CD25 Negative	CD25 Positive	Mann-Whitney test	
	Median (IQR)	Median (IQR)	Z	P-value
CD34 (%)	39.5 (2 – 82)	2.5 (1.5 – 31)	-1.075	0.282
CD123 (%)	15.2 (8.58 – 23.9)	34.07 (27.77 – 43.45)	-2.562	0.01*
CD123 CD34 coexpression (%)	5.65 (0 – 18.9)	0.2 (0 – 15.26)	-0.746	0.456
CD123 CD25 coexpression (%)	0.08 (0 – 0.34)	21.11 (16.31 – 23.52)	-3.193	0.001
TLC ($x10^3$/µl)	29.55 (5.1 – 70)	67.65 (56.55 – 92.65)	-1.556	0.12
Hb (g/dl)	6.9 (5.6 – 8)	6.05 (5.5 – 6.5)	-1.221	0.222
PLT ($x10^3$/µl)	34 (21 – 61)	133.5 (60.5 – 164.5)	-1.22	0.222
PBB (%)	45 (20 – 65)	73.5 (55 – 88.5)	-1.593	0.111
BM Blast cells (%)	72.5 (50 – 83)	82.5 (77.5 – 90)	-1.531	0.126
Age (yrs) [mean+ SD)	44.0 • ± 16.7	44.0 • ± 20.9	(t-test)-0.000	1.000

TLC: Total leucocytic count; Hb: Hemoglobin; PBB: Peripheral blood blast cells percentage; PLT: Platelet; BM: Bone marrow; N: number; *: Significant p value

Table (4): Relation between Outcome and qualitative parameters:

		Outcome						Test of significance	
		Non Remission		Remission		Death			
		N	%	N	%	N	%	X^2	P-value
Sex	**Female**	6	60.00	5	41.67	6	75.00	2.296	0.317
	Male	4	40.00	7	58.33	2	25.00		
Hepatomegaly	**Negative**	8	80.00	8	66.67	6	75.00	0.513	0.774
	Positive	2	20.00	4	33.33	2	25.00		
Slpeenmegaly	**Negative**	8	80.00	9	75.00	5	62.50	0.706	0.703
	Positive	2	20.00	3	25.00	3	37.50		
Lympadenopathy	**Negative**	8	80.00	10	83.33	7	87.50	0.184	0.912
	Positive	2	20.00	2	16.67	1	12.50		
FAB subtype	**M0**	1	10.00	0	0.00	0	0.00	18.416	0.048*
	M1	1	10.00	0	0.00	2	25.00		
	M2	3	30.00	6	50.00	1	12.50		
	M3	0	0.00	4	33.33	1	12.50		
	M4	4	40.00	1	8.33	1	12.50		
	M5	1	10.00	1	8.33	3	37.50		
Cytogenetic studies (conventional karyotyping, FISH)	**Normal**	4	50.00	4	36.36	6	75.00	16.698	0.033*
	t(8;21)	0	0.00	3	27.27	0	0.00		
	inv 16	3	37.50	1	9.09	0	0.00		
	t(15;17)	0	0.00	3	27.27	1	12.50		
	11q23	1	12.50	0	0.00	1	12.50		
Cytogentic prognostic groups	**Unfavorable**	1	12.50	0	0.00	1	12.50	6.776	0.148
	Intermediate	4	50.00	4	36.36	6	75.00		
	Favorable	3	37.50	7	63.64	1	12.50		
CD 34 expression	**Negative**	3	25.00	5	41.67	4	33.33	0.772	0.680
	Positive	7	38.89	7	38.89	4	22.22		
CD25 expression	**Negative**	10	38.46	12	46.15	4	15.38	12.470	0.002*
	Positive	0	0.00	0	0.00	4	100.00		
CD123 expression	**Negative**	2	11.76	12	70.59	3	17.65	20.461	<0.001**
	Positive	8	61.54	0	0.00	5	38.46		
$CD123^+CD25^+$ coexpression	**Negative**	10	100.00	12	100.00	4	50.00	12.470	0.002*
	Positive	0	0.00	0	0.00	4	50.00		
$CD123^+CD34^+$ Coexpression	**Negative**	4	17.39	12	52.17	7	30.43	11.693	0.002*
	Positive	6	85.71	0	0.00	1	14.29		

N: number; *: Significant p value; **: Highly significant p value

Table (5): Relation between Outcome and quantitative parameters:

	Remission	**Non remission**	**Death**	**Kruskall-Wallis test**	
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**K**	**P-value**
CD123 (%)	11.55 (7.87 – 15.1)	24.16 (21.2 -35.2)	27.77 (11.56 –37.95)	8.794	0.012*
CD123 CD34 co expression (%)	4.82 (0 – 10.6)	24.16 (0 – 25)	1.14 (0 – 10.12)	2.412	0.299
CD25(%)	0.1 (0.06 – 0.22)	0.04 (0 – 0.06)	17.5 (0.02 – 22.54)	4.698	0.095
CD123 CD25 co expression (%)	0.17 (0.07 – 0.48)	0.03 (0 – 0.06)	7.15 (0.11 – 21.11)	6.073	0.048*
TLC	7.9 (4.05 – 45.8)	58.95 (19.5 – 157)	56.55 (26.45 –92.65)	3.283	0.194
Hb	7.25 (6.2 – 8.25)	6.6 (5.1 – 7.9)	6.05 (5.3 – 7.45)	1.93	0.381
PLT	36.5 (22.5 – 47.5)	32.5 (18 – 61)	91.5 (23 – 164.5)	1.726	0.422
PBB (%)	45 (16.5 – 60)	46 (10 – 79)	62.5 (35 – 85.5)	1.629	0.443
BM Blast cells(%)	65 (39.5 – 80)	80 (70 – 85)	81 (72 – 89)	3.727	0.155
Age (yrs) [mean±SD)	45.3 ±13.6	43.8 ±17.8	42.3 ± 21.8	(F) 0.076	0.927

TLC: Total leucocytic count; Hb: Hemoglobin; PBB: Peripheral blood blast cells percentage; PLT: Platelet; BM: Bone marrow; N: number; *: Significant p value