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CD133 Expression in Adult Egyptian Acute Leukemia Patients and its Impact on Disease Outcome

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ABSTRACT

Background: CD133 antigen is expressed restrictively by the immature subset of the CD34+ cells; hence it is expected to be a valuable prognostic marker in acute leukemia.

Aim: To assess CD133 expression frequency in patients with acute leukemia and to evaluate its relation to disease outcome.

Patients and Methods: The present study was carried on seventy-five newly diagnosed acute leukemia patients, recruited from hematology/oncology clinic of National Cancer Institute. The patients were divided into two groups, 24 acute lymphoblastic leukemia (ALL) and 51 acute myeloblastic leukemia (AML) patients. Patients were followed-up through out the period of the study (12 months). All patients were subjected to the complete history taking, thorough clinical examination and laboratory investigations, including complete blood count (CBC), Bone marrow (BM) aspirate examination, Immunophenotyping and assessment of CD133 expression using EPICS XL Flow Cytometer.

Results: CD133 was expressed in 28/75 patients (37.3%), 21/51 patients with AML (41.17%) and 7/24 patients with ALL (29.16%). The expression of CD133 was higher in AML than ALL without statistical significance (20.41±19.28 vs. 15.2±14.69; p=0.20). No significant correlation was found between CD133 +ve expression and the clinical data as regard sex, age, hepatomegaly, splenomegaly and lymphadenopathy in both ALL and AML. No significant correlation was found between CD133 +ve expression and the hematological data as regard, WBCs, Hb, platelets and peripheral blasts in both ALL and AML. There was significant positive correlation between CD133 and BM blasts in ALL (r=0.45; p=0.02), but insignificant in AML. In both ALL and AML, patients with CD133 +ve expression had statistically significant poor clinical outcome (relapse or death) (p=0.012 and 0.021). Patients with CD133+ve expression had shorter overall survival compared with CD133 –ve; this was significant in AML (8.95±0.69 vs. 10.3±0.54 months, p=0.05) and insignificant in ALL.

Conclusion: CD133-positive expression is a poor prognostic factor in adult acute leukemia and its expression could characterize a group of acute leukemic patients with resistance to standard chemotherapy, as well as high incidence of relapse and death. The use of CD133 as a prognostic marker in acute leukemia would be recommended to offer a chance for early intensive therapeutic intervention in cases designated as having poor prognosis.

Key Words: *AML – ALL – CD133.*

INTRODUCTION

Leukemia arises through the acquisition of genetic mutations in hematopoietic stem or progenitor cells, resulting in impairment of hematopoietic and unrestrained proliferation of an immature clone. The condition is lethal within a few months without treatment, but most young patients reach complete remission with chemotherapy. Many of them will relapse after a while, but an increasing number of young people survive for a long time **[1]**. Assessment of the prognosis of acute leukemia involves a number of clinical and laboratory criteria such as morphology, surface markers, cytogenetics and other recent criteria such as transcription factors and cytokines **[2,3]**. Prominin (CD133) is a membrane protein consisting of five membrane-spanning domains, two large N-glycosylated extracellular loops, an extracellular N-terminal domain and acytoplasmic C-terminal domain **[4]**. It has been initially isolated as a cell surface marker expressed on a subpopulation of CD34+ cells in hematopoietic stem and progenitor cells derived from human fetal liver, bone marrow and peripheral blood **[5,6]**. CD133 has been shown to be expressed in both acute and chronic myeloid leukemia and lymphoblastic leukemia in both adults and pediatrics **[7]**. Several studies

indicated that the CD133 antigen expression is related to CD34 cell surface expression may provide alternative, but similar information with regards to leukemic blast phenotype in acute myeloid leukemia (AML) **[8,9]**. However, in acute lymphoblastic leukemia (ALL), encouraging preliminary observations suggested that CD133 may provide an important marker capable of distinguishing normal stem progenitors from lymphoid leukemia initiating blasts **[10,11]**. The present study aimed to assess CD133 expression frequency in patients with acute leukemia and to evaluate its relation to disease outcome.

PATIENTS AND METHODS

Patients:

The present study included 75 newly diagnosed acute leukemia patients, recruited from hematology/oncology clinic of the National Cancer Institute, Cairo University. The study was approved by the Institutional Review Board (IRB) and a written informed consent was obtained from all cases before participation in the study. Patients were divided into two groups, 24 ALL (15 males and 9 females), their ages ranged from 18 to 65 with a mean of 37.12± 12.59 and a median of 34.5 years, and 51 AML (25 males and 26 females), their ages ranged from 25 to 83 with a mean of 40.87±16.3 and a median of 40 years. Patients were followed-up through out the period of study (12 months).

All cases were subjected to the following:

1- Complete history taking and thorough clinical examination.

2- Laboratory investigations:

Complete blood count (CBC) was done by the use of cell dyne-3700 (Abbott Diagnostics, Dallas, USA) with examination of peripheral blood (PB) stained smears for differential leucocytes count and blast cells percentage. BM aspiration and examination of stained smears was performed. Immunophenotyping and assessment of CD133 expression as well as CD133/CD34 co expression in BM or PB samples were evaluated by the use of EPICS XL Coulter Flow Cytometer (Coulter-USA), (Fig. 1). Diagnosis of acute leukemia was based on the presence of blast cells ≥20% in BM film according to WHO proposal, together with presence of immunophenotyping results consistent with AML and ALL. Assessment of remission achievement was done after the induction of therapy by BM on day 14 and day 28 as well as follow-up of the patients through out the period of study with a range of 1 to 12 months and a median of 12 months.

Methods:

Fresh PB or BM samples were kept at ambient temperature and processed for immunophenotyping within 6 hours of collection. The EDTA anticoagulated BM and PB samples were diluted with phosphate buffered saline (PBS), pH 7.4 (Sigma Chemicals, St Louis), the final cell count suspension was adjusted at $10x10^3$/ml. For each sample, a set of tubes was prepared for a panel of fluorescein isothiocyanate (FITC)/phycoerythrin (PE) conjugated Mo-Abs used for diagnosis of acute leukemia including one for the isotypic matched negative control (supplied by Beckman Coulter, France), B-lineage markers (CD19, CD22, CD10), T-cell markers: (CD2, CD3, CD5, CD7), Myeloid markers: (CD13, CD33 and intracellular MPO), CD61, CD 41 and glycophorin in suspected cases. Monocytic marker: (CD14), and common progenitor's markers: (CD34, HLA-DR). PE labeled MoAb for detection of CD133 (Miltenyi Biotec, Germany).

Immunophenotypic analysis:

Immunophenotypic analysis was performed using whole blood staining method. Fresh samples were obtained from peripheral blood or bone marrow at the time of diagnosis. Double and Triple marker labeling was performed, including proper isotype controls. Samples were analyzed on Flow Cytometer (Coulter Epics, XL, Hialeh).

Detection of surface markers by direct staining:

The whole blood staining method was performed **[12]**. In short, 10µl labeled Mo Ab was added to 100µl whole blood (or BM), incubated in the dark for 20 minutes, hemolyzed by Lysing solutions, A11894, Beckman Coulter) and washed by PBS then analyzed.

Detection of intracellular markers:

Hundred µl of whole blood was lysed using lysis solution (Becton & Dickinson) for 10 minutes. Cells were washed once and re-suspended in 1ml PBS. A mixture of 500µl 4% paraformaldehyde as fixative, 500µl PBS and 5µl tween 20 as detergent was added to the cells

and incubated for 10min. The cells were washed and 10µl Mo Ab was added and incubated for 30min. at 4ºC. Cells were washed, re-suspended in 500 µl PBS and analyzed **[12]**.

A minimum of 10000 events were acquired. Blast cell population was selected based on its forward and side scatter properties. The percentage of blast cells positive for the relevant studied marker was determined as a percentage from the blast cells population. The negative isotypic control was set at 0.5%. Cells were considered positive for a certain marker when ≥20% of cells expressed it, except for CD34 and intracellular MPO where its expression by 10% of cells was sufficient to confer positivity.

Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS-version 17). All data was expressed as mean±SD. For statistical evaluation, Student *t*-test was used. Significance was accepted at $p \leq 0.05$. Qualitative data were described in the form of number and percentage. Correlation Coefficient (*r*) was used for showing positive and negative correlation between variables. Quality of survival between studied groups was tested by Kaplan-Meier curve.

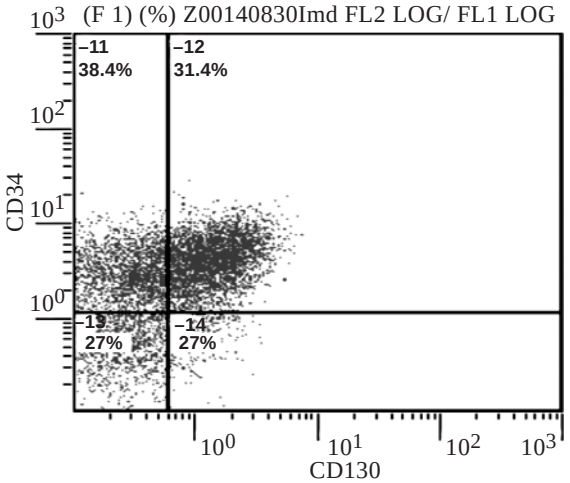

Fig. (1): Histogram showing co-expression of CD34 and CD133.

RESULTS

The present study was carried out on 75 newly diagnosed acute leukemia, 24 ALL and 51 AML. The demographic, clinical and hematological data of the studied ALL and AML patients are presented in (Tables 1,2).

Table (1): Demographic and clinical data of 75 acute leukemia patients.

Parameter	ALL (No.=24)	AML (No.=51)
Age:		
Mean±SD	37.12±12.59	40.87±16.3
Range	18-62	18-83
Median	34.5	40
Gender: No (%):		
Male	15 (62.5)	25 (49)
Female	9 (37.5)	26 (51)
Hepatomegaly:		
No. (%)	8 (33.3)	231 (60.78)
Splenomegaly:		
No. (%)	8 (33.3)	29 (56.86)
Lymphadenopathy:		
No. (%)	17 (70.8)	21 (41.18)

ALL : Acute lymphoblastic leukemia.
AML : Acute myeloid leukemia.

Table (2): Hematological data of 75 acute leukemia patients.

Parameter	ALL (No.=24)	AML (No.=51)	*p*
TLC: $x10^9$/L	46.2±45.57 (1.13-273)*	39.5±38.59 (1.1-252)	0.5
Hb: g/dl	7.6±1.47 (5.3-12)	7.8±3.22 (2.6-25)	0.8
Platelets: $x10^{12}$/L	67.16±57.6 (5-256)	52.68±52.29 (2-260)	0.2
PB blasts	85±13.1 (44-97)	71.0±15.2 (33-97)	<0.001
BM blasts	78±20.1 (5-98)	79.0±12.9 (45-98)	0.824

ALL : Acute lymphoblastic leukemia.
AML : Acute myeloid leukemia.
***** Mean±SD (range).

CD34 was expressed in acute leukemia with higher expression in ALL than AML (42.97%± 32.09 vs 27.63%±26.71, p=0.046). CD133 was positive in 28/75 (37.3%) patients with acute leukemia, 21/51 (41.17%) with AML and 7/24 (29.16%) with ALL, its expression was insignificantly higher in AML than ALL (20.41± 19.28 vs 15.2±14.69; p=0.20). CD133 expression was restricted only to CD34 positive cells; all CD133 positive cells expressed CD34 (Fig. 1).

There was positive correlation between CD34 and CD133, significant in ALL (Fig. 2) and insignificant in AML patients (r=0.55, p= 0.001 and r=0.15, p=0.27 respectively).

Both groups showed no significant correlation between CD133 on one side and clinical data as regards sex, age, hepatomegaly, splenomegaly

or lymphadenopathy on the other side (Table 4). As regards hematological parameters significant positive correlation was encountered between CD133 and BM blasts in ALL and insignificant in AML (r=0.45; p=0.02 and r=0.04, p=0.78 respectively), other parameters showed insignificant correlations in both groups.

As regards the clinical outcome the percentage of remission was significantly higher among patients with CD133 negative than patients with CD133 positive expression in both groups (p= 0.012, 0.021). On the other side, the percentage of relapse and death was significantly higher in patients with CD133 positive than patients with CD133 negative in both groups (p=0.012, 0.021, Table 3).

Table (3): Impact of CD133 expression on the outcome of 75 acute leukemia patients.

Parameter	No.	Remission		Relapse		p value
		No.	%	No.	%	
ALL	24	17	70.8	7	29.2	
CD133 –ve	17	14	82.35	3	17.65	0.012
CD133 +ve	7	3	42.9	4	57.1	
AML	51	30	58.8	21	41.2	
CD133 –ve	30	22	73.33	8	26.67	0.021
CD133 +ve	21	8	38.1	13	61.9	

Patients with positive CD133 expression had shorter overall survival (OS) compared to CD133-negative patients; the difference was significant in AML (8.95±0.69 vs. 10.3±0.54 months, p=0.05, Fig. 3) and insignificant in ALL (9.16±0.77 vs. 10.6±1.67 months, p= 0.3, Fig. 4).

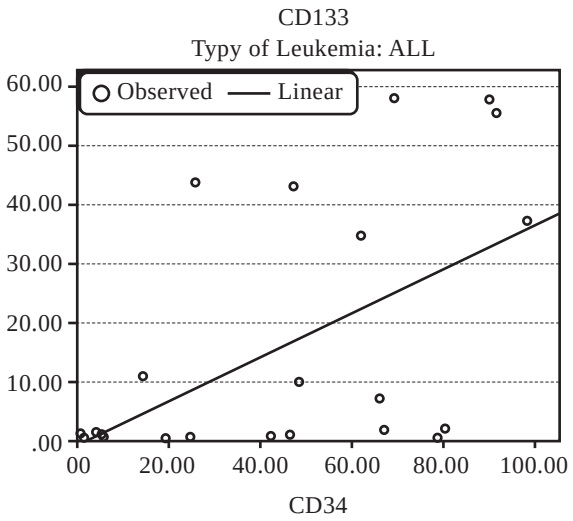

Fig. (2): Correlation between CD133 and CD34 in ALL.

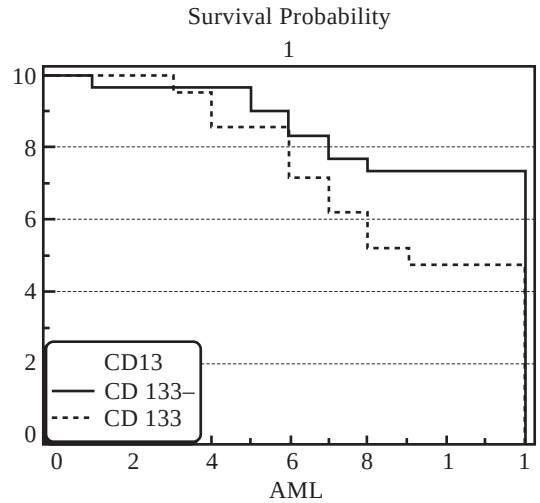

Fig. (3): Kaplan-Meier survival curve comparing survival in CD133 negative cases and CD133 positive in AML patients.

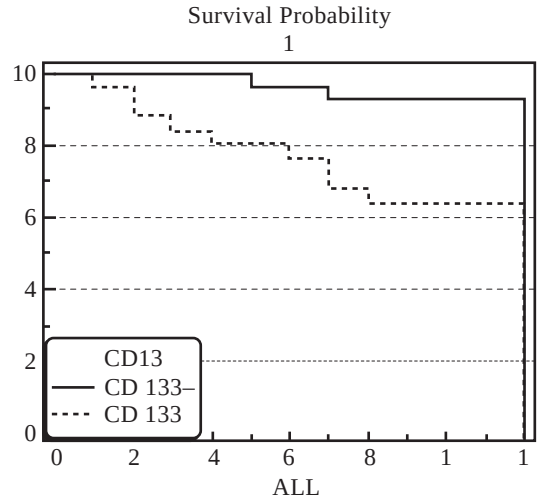

Fig. (4): Kaplan-Meier survival curve comparing survival in CD133 negative cases and CD133 positive in ALL patients.

DISCUSSION

Leukemia arises through the acquisition of genetic mutations in hematopoietic stem or progenitor cells, resulting in impairment of normal hematopoiesis and unrestrained proliferation of an immature clone. The condition is lethal within a few months without treatment, but most young patients reach complete remission with chemotherapy. Many of them will relapse after a while, but an increasing number of young people survive for a long time **[1]**.

Given the fact that CD133 antigen is expressed restrictively in the more immature subset of the CD34 cell population, we could expect

the CD133 antigen to be more valuable prognostic marker compared to the CD34 antigen. It has also been suggested that, like the CD34 antigen, expression of the CD133 antigen in acute leukemia could be correlated with either a more immature phenotype of the blast population or to a bad prognosis **[13]**.

In this study CD34 showed higher expression in ALL than AML with statistical significance; this result is in agreement with Filler **[9]**, who reported that CD34 antigen is expressed in a relatively high proportion of cases ranging from 30 to 60% in AML and from 60 to 70% in ALL, and that its presence has been related to more immature morphological and immunophenotypic features, as well as a poorer prognosis.

In this study, CD133 was expressed in 37.3% of acute leukemia; 41.17% in AML and 29.16% in ALL groups. These results are in agreement with previous reports **[8,14-17]**. However Cox and coworkers **[7]** stated that there have been conflicting reports on the expression of AC133 in ALL, whereas some found high level of CD133 in particular cases **[8-11]**, others detected only low levels **[5]** or none at all **[13]**. The frequency of CD34 expression was proved to be found on 100% of CD133-positive cases, this result confirmed that CD133 expression is restricted to CD34 positive cells and this is in agreement with previous reports **[14,19]**. We detected positive correlation between CD133 and CD34 expression significant in ALL and insignificant in AML; this is in agreement with previous reports **[18,19]**.

To elucidate the value of CD133 expression as a prognostic factor in acute leukemia, we investigated the significance of its expression in relation to various clinical, laboratory and standard prognostic factors, as well as to clinical outcome of patients. No significant correlation was noted between age of patients and positivity of CD133 expression in acute leukemia patients, this is in agreement with previous reports **[13-17]**. In contrast to our study Wuchter and coworkers **[8]** demonstrated that there was a weak inverse correlation between CD133 positive expression and age of ALL patients.

In our study we found that no significant association was detected between the gender and the CD133 positive expression, which is in agreement with previous results **[13-16]**. No association was detected between clinical variables (hepatomegaly, splenomegaly and lymphadenopathy) and CD133 expression; this is in agreement with other reports **[10,14-16]**.

Several studies reported that there were insignificant correlation between CD133 positive expression and hematological data of patients with acute leukemia **[10,14,16,18-20]**, those results are in agreement with our result as regards hemoglobin concentration, WBCs, platelet counts and percentage of leukemic blast cells in PB, but in contrast we found that there was significant positive correlation with BM blasts in ALL. Elgendi et al., **[15]**. Reported that there was significant positive correlation between CD133 positive expression and higher percentage of BM and peripheral blasts and this is in partial agreement with our results.

As regards outcome, we found that the percentage of remission among patients with CD133 negative was significantly higher than patients with CD133 positive expression in both ALL and AML. The other way round, the percentage of relapse and death in patients with CD133 positive was significantly higher than patients with CD133 negative in both groups. These results are consistent with previous reports who found a trend towards higher complete remission (CR) rates in CD133-negative acute leukemia cases when compared to CD133-positive ones **[15,16,20]** and a tendency for poorer outcomes in CD133-positive acute leukemia compared to CD133-negative ones **[13,15]**.

In the current study, the unfavorable prognosis conferred by CD133 expression was also reflected on the OS for patients. This was previously reported by some authors who proved the association between CD133 expression and shorter remissions in acute leukemia patients **[13,15,16,20]** but denied by others who reported that the increased expression of this protein was not significantly associated with a shorter OST **[10,14,23]**. This discrepancy between the studies could be attributed, at least partially, to the difference in the methods of detection of CD133 and the therapeutic protocols applied to the patients.

In conclusion, CD133 expression was highly associated with poor prognosis in acute leukemia patients. It may be considered an adverse prognostic factor; its expression could characterize

a subgroup of acute leukemia patients with higher resistance to standard chemotherapy, relapse or death. Accordingly, it is recommended to add CD133 to acute leukemia workup panel to offer a chance for early intensive therapeutic intervention in cases designated as having poor prognosis.

REFERENCES

1- Tangen JM, Fløisand Y, Foss-Abrahamsen J, et al. Survival in adults with acute myelogenous leukemia. Tidsskr Nor Laegeforen. 2008; 128 (10): 1164-1167.

2- Whitlock JA, Gaynon PS. Acute lymphocytic leukemia. In: Lee GR, Foerster J, Lukens J, et al. eds. Wintrobe's Clinical Hematology. 10th ed. Baltimore: Williams & Wilkins Company. 1999; 2241-2271.

3- Roman-Gomez J, Jimenez-Velasco A, Castillejo JA, et al. Promoter hypermethylation of cancer-related genes: A strong independent prognostic factor in acute lymphoblastic leukemia. Blood. 2004; 104: 2492-2498.

4- Corbeil D, Rper K, Fargeas CA, et al. Prominin astory of cholesterol, plasma membrane protrusions and humanpathology, Traffic. 2001; 2: 82-91.

5- Miraglia S, Godfrey W, Yin AH. et al. A novel five transmembrane hematopoietic stem cell antigen: Isolation, characterization, and molecular cloning, Blood. 1997; 90: 5013-5021.

6- Li CW, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007; 67: 1030-1037.

7- Cox CV, Diamanti P, Evely RS, et al. Expression of CD133 on leukemia-initiating cells in childhood ALL. Blood. 2009; 113: 3287-3296.

8- Wuchter C, Ratei R, Spahn G, et al. Impact of CD133 (AC133) and CD90 expression analysis for acute leukemia immunophenotyping. Haematologica. 2001; 86: 154-161.

9- Feller N, Kelder A, Westra G, et al. Positive selection for CD90 as a purging option in acute myeloid leukemia stem cell transplants. Cytometry B Clin Cytom. 2008; 74: 9-16.

10- Vercauteren SM, Sutherland HJ. CD133 expressionon AML cells and progenitors. Cytotherapy. 2001; 3: 449-459.

11- Buhring HJ, Seiffert M, Marxer A, et al. AC133 antigenexpression is not restricted to acute myeloid leukemia blastsbut is also found on acute lymphoid leukemia blasts and on asubset of CD34+ B-cell precursors. Blood. 1999; 94: 832-833.

12- Jung RT, Schauer U, Heusser C, Neumann C, Rieger C. Detection of intracellular cytokines by flow cytometry. Immunol. Method. 1993; Feb 26; 159 (1-2): 159-197.

13- Horn PA, Tesch H, Staib P. Expression of CD133, a novelhematopoietic precursor antigen, on acute myeloid leukemia cellline. Blood. 1999; 93: 1435-7.

14- Lee ST, Jang HJ, Min YH, et al. AC133 antigen as aprognostic factor in acute leukemia. Leuk Res. 2001; 25: 757-761.

15- Elgendi HM, Mekawy MA, Abdel Wahab SE, et al. AC133 Expression in Egyptian children with Acute Leukemia: Impact on treatment response and disease outcome. J Pediatr Hematol Oncol. 2010; 32: 286-293.

16- Tolba FM, Foda ME, Kamal HM, et al. Expression of CD133 in acute leukemia. Med Oncol 2013; 30: 527-534, DOI 10.1007/s12032-013-0527-6.

17- Guenova M, Balatzenko G. CD133-2 (AC141) expression analysisin acute leukemia immunophenotyping in correlation toCD34 and P-glycoprotein. Hematology. 2008; 13 (3): 137-141.

18- Wang W, Wang HY, Zhao HX, et al. Expression of CD133 in bone marrow cells of patients with leukemia and myelodysplastic syndrome. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2007; 15 (3): 470-473.

19- Fauth F, Weidmann E, Martin H, et al. AC133 Expression on acute myeloid leukemia blasts: Correlation to FAB and to CD34 expression and possible implications for peripheral blood progenitor cell purging in AML. Leuk Res. 2001; 25: 191-196.

20- Zhou Y, Li Q, Meng HX, et al. The expression andclinical significance of early differentiation antigens in acute leukemia. Zhonghua Nei Ke Za Zhi. 2005; 44: 46-49.

21- Kim JH, Cho HI, Kim EC, et al. A study on 289 consecutive Korean patients with acute leukaemias revealed fluorescencein situ hybridization detects the MLL translocation without cytogenetic initially and during follow-up. Br J Haematol. 2002; 119: 930-939.

22- Taguchi J, Miyazaki Y, Tsutsumi C, et al. Expression of the myeloperoxidase gene in AC133 positive leukemia cells relatesto the prognosis of acute myeloid leukemia. Leuk Res. 2006; 30 (9): 1105.

23- Lichtman MA, Liesveld JL. Acute Myelogenous leukemia. In: Lichtman MA, Kipps TJ, Kaushansky K, Beutler E, Seligsohn U, Prchal J editors. Williams Hematology. 7th ed. USA; McGraw-Hill Companies. J Immunol Methods. 2006; 1183.