

Relationship between the Expression of CD34, CD123 and Myeloperoxidase Markers by Flow Cytometry and Response to Induction Therapy in Acute Myeloid Leukemia

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Abstract

Background Different immunophenotypic markers were found to be related to the prognosis of acute myeloid leukemia. Among them are CD34, CD123 and myeloperoxidase.

Objective To evaluate the relationship between the expression of CD34, CD123 and myeloperoxidase markers by flow cytometry; and the initial response to induction therapy in acute myeloid leukemia patients.

Method A cohort of forty one patients with newly diagnosed *de novo* acute myeloid leukemia were prospectively tested for the expression of CD34, CD123 and myeloperoxidase using multicolor flow cytometry and re-evaluated for the response to a 7+3 induction therapy regimen.

Results It was found that 64.29% of CD123⁻ patients achieved complete remission while 70.37% of CD123⁺ patients not ($P = 0.035$). For CD34, 55.56% of CD34⁻ patients achieved complete remission while 63.64% of CD34⁺ cases not. The induction failure in CD34⁺M3 cases was 100% ($P = 0.045$). Regarding myeloperoxidase, 61.54% of patients who had >20% myeloperoxidase expression achieved complete remission while 70.37% (myeloperoxidase expression in <20% of cells) failed to achieve complete remission ($P = 0.05$).

Conclusion Expression of CD34 and CD123 and weak expression of myeloperoxidase (<20% of blast cells) are associated with poor response to induction therapy in acute myeloid leukemia patients.

Keywords Flow cytometry, CD34, CD123, myeloperoxidase, acute myeloid leukemia

List of Abbreviation: AML = Acute myeloid leukemia, HSCs = hematopoietic stem cells, MPO = Myeloperoxidase, PMNs = polymorphonuclear cells, ALL = acute lymphoblastic leukemia, SBB = Sudan Black B, WBC = white blood cell count.

Introduction

Acute myeloid leukemia (AML) is a clonal, malignant disease of hematopoietic tissues that is characterized by accumulation of leukemic blast cells, principally in the marrow, that impair the production of normal blood cells leading to neutropenia, anemia and thrombocytopenia⁽¹⁾. Iraq is among the world countries with both high incidence and low survival rate of AML⁽²⁻⁴⁾.

The clinical outcome of acute myeloid leukemia (AML) is extremely variable, ranging from survival of a few days to cure. Different clinical and biological features at diagnosis have been reported as useful for the prediction of clinical outcome⁽⁵⁾.

CD34 is a member of the CD34 family of cell-surface transmembrane proteins⁽⁶⁾. It's expressed on the most immature hemopoietic stem cells. It may play a role in the attachment of stem cells to the bone marrow extracellular matrix or to stromal cells⁽⁷⁾. In addition, it has a signal transducing capacity, causing actin

polymerization⁽⁸⁾ and it's widely used for the identification and isolation of hematopoietic stem cells (HSCs) and progenitors⁽⁹⁾.

High expression of CD34 has been linked to poor prognosis in acute myeloid leukemia patients⁽¹⁰⁾. Interleukin-3 receptor subunit alpha or CD123 is a single-pass type I membrane protein which belongs to the type I cytokine receptor family and type 5 subfamily. CD123 is strongly expressed in various leukemic blasts and leukemic stem cells although it is not expressed by normal hemopoietic stem cells.

CD123 seems to be an excellent target for the therapy of leukemia^(11,12). Its ligand is interleukin-3 which is a multipotent cytokine that promotes the development of hemopoietic progenitors into cells of the erythroid, myeloid and lymphoid lineages⁽¹²⁾, and induces blast cells formation⁽¹³⁾.

Myeloperoxidase (MPO) is a heme-containing peroxidase highly expressed by polymorphonuclear neutrophils. Its major function is generation of hypochlorous acid as part of the neutrophil's antimicrobial armory⁽¹⁴⁾. It is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in polymorphonuclear cells (PMNs)⁽¹⁴⁾.

The demonstration of peroxidase in at least 3% by cytochemistry or 10% by flow cytometry of bone marrow blasts defines an acute leukemia as AML⁽¹⁵⁾.

Anti-MPO antibodies had shown consistent negative results in acute lymphoblastic leukemia (ALL) cases^(15, 16).

Anti-MPO has an important role in distinguishing minimally differentiated AML (M0) and biphenotypic acute leukemia from acute unclassified leukemia (AUL) and ALL even when CD13 and CD33 are negative⁽¹⁷⁾.

The objective of the current study is to clarify the relation between the expression of CD34, CD123 and MPO measured by multiparametric flow cytometry and the response to induction therapy in acute myeloid leukemia in Iraqi patients.

Methods

This prospective cohort study was conducted on 41 patients older than 15 years with newly diagnosed *de novo* AML. Patients were taken from the National Center of Hematology and Baghdad Teaching Hospital, between February and July 2013. This research was approved by the Ethical Committee at the College of Medicine, Al-Nahrain University. Signed informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

Patients suspected to have acute myeloid leukemia were subjected to further investigations. About 2.5 ml of peripheral blood and about 0.5 ml of bone marrow aspirate was collected in K2-EDTA tube from each patient. The hematological parameters were obtained by automated hematology analyzer (CELLDYN Ruby). Peripheral blood and bone marrow films were stained with Leishman and Sudan Black B (SBB) stains. After morphological diagnosis of AML in the hematology laboratory of the national center of hematology and the teaching laboratories in the medical city, samples were sent for flow cytometry.

After the morphological diagnosis was established in the hematology laboratory, the specimen was transferred within 24 hours to a private laboratory where the flow cytometric markers specific to this study were tested.

Multi-color immunophenotyping by Partec Cyflow Cube 6 flow cytometer and interpretation of markers by FACS Express 4 software was done at the private lab. Anti-CD45 and Anti-CD34 reagents from Partec Company; and anti-CD123 and anti-MPO from BD Company were used. Staining for the surface anti-CD123, anti-CD45 and anti-CD34 was accomplished in one tube, while staining for the cytoplasmic anti-MPO was done in a separate tube.

Blasts and leukemia cells were gated according to the expression of CD45 and side scatter. Cell population with low side scatter and negative or dim expression of CD45 were regarded as the blast cells or leukemia cells. From this population of cells, the expression of CD123 and

CD34 was calculated in comparison to the isocontrol. On the other hand; the gating for MPO was done according to the physical properties (forward scatter FSC/side scatter SSC) in comparison with the normal lymphocytes in the same blood or bone marrow sample (internal control)⁽¹⁸⁻²⁰⁾.

Patients received 7+3 regimen containing cytarabine, given continuously for seven days through an intravenous line. Daunorubicin, was given in a single IV dose for the first three days of treatment. Patients with French American British (FAB) M3 received, in addition to the drugs mentioned, All-transretinoic acid 45 mg/m² divided into two doses and given orally. Older patients (> 60 years old) received cytarabine at 100 mg/m² for 5 days⁽²¹⁾.

All patients were followed up for assessment of response to the first course of induction therapy. The same procedure of peripheral blood and bone marrow collection and processing mentioned above was done after approximately 4 weeks after the start of therapy.

Patients were classified into complete remission (Bone marrow blasts < 5%; absence of blasts with Auer rods; absolute neutrophil count > 1.0 X10⁹/L; and platelet count > 100 X10⁹/L), resistant disease (persistent leukemia by blood and/or bone marrow examination) and death during induction according to the Cheson criteria of response⁽²¹⁾. For statistical purposes, the last two groups were gathered into one group, the non-remission group.

All statistical operations were done by Microsoft excel 2010 and SPSS programs. The *P* value for significance was calculated from Chi square test and Fisher Exact test for 2X2 contingency tables when the Chi square test was not applicable.

A 20% cut-off value was put to indicate the positivity of surface markers CD34 and CD123; while the cut-off value for the cytoplasmic marker myeloperoxidase was 10%. A cut-off value (20%) for MPO was assessed for prognostic significance⁽²⁰⁾.

Results

Forty one patients were enrolled in the study; the mean age was 38.7±17.23 years (mean±SD), the range was 16-78 years. Highest incidence was reported at the age group 15-24 years while lowest at > 65 years. The mean WBC count at presentation was 51.3 X10⁹/l, and ranged from 1.1-230 (X10⁹/L). Seven patients (17.07%) were leukopenic, four patients (9.75%) had normal WBC count, while the majority of cases (30 patients, 73.17%) had leukocytosis (table 1 and figure 1).

FAB M3 formed the major portion of AML subtypes (26.83%), followed by M2 and M5 (19.51% for each) then M1 and M4 (17.07% for each). M0, M6 and M7 subtypes were not represented in the current study (Table 1). Because of a technical error; one sample was not tested for MPO marker. Thirty six of 40 patients who were tested for MPO showed positive results (90%). All the other four negative cases were M5 subtype. Regarding CD34 marker, 33 cases (80.49%) were positive; while CD123 was positive in 27 patients (65.85%) as shown in table 2.

The peak of CD34 expression was detected in M4 subtype (100%), while its expression was poor in M3 subtype (45.45 %). CD123 was mostly expressed in M1 (85.72 %) and least expressed in M3 (54.54%) subtypes.

At the end of induction therapy; twelve patients (29.26%) died during the first 4 weeks of induction therapy, twelve patients (29.26%) did not achieve the morphological criteria of complete remission, and seventeen patients (41%) achieved complete remission by examination of their bone marrow after about 4 weeks of treatment.

Out of the 27 patients who were CD123⁺, only 8 (29.63%) achieved complete remission, in contrast to the rest 19 (70.37%). On the other hand, 9 (64.28%) of the 14 patients who were CD123⁻ achieved CR, while the rest 5 (35.72%) failed to achieve response (Table 3 and fig. 1).

Table 1. Distribution of AML patients according to gender, WBC count and FAB subtype

Variable		Number (%)	Range	Mean±SD
Gender	Male	24 (58.54)	N/A	N/A
	Female	17 (41.46)		
WBC count (X10 ⁹ /l)	< 4	7 (17.07)	1.1-230	51.3±55.5
	4-11	4 (9.76)		
	> 11	30 (73.17)		
Hemoglobin (g/dl)	Low*	40 (97.56)	3.48-12.7	8.48±2.19
	Normal	1 (2.44)		
Platelet count (X10 ⁹ /l)	Low**	39 (95.12)	12-396 X10 ⁹ /l	63.07±74.03
	Normal	2 (4.88)		
FAB Subtype	M0	0 (0)	N/A	N/A
	M1	7 (17.07)		
	M2	8 (19.51)		
	M3	11 (26.83)		
	M4	7 (17.07)		
	M5	8 (19.51)		
	M6	0 (0)		
	M7	0 (0)		

*Low hemoglobin if less than 13 g/dl in men and 12 g/dl in women, **Low Platelet if less than 150X10⁹/l (men and women)

Table 2 Flow cytometric expression of the studied markers

Parameter	+ve	%	-ve	%	Median	Mean±SD	Total
CD123	27	65.85	14	34.15	27.59	35.31±22.62	41
CD34	33	80.49	8	19.51	29.26	29.46±17.99	41
MPO	36	90	4	10	18.07	22.37±17.88	40

Thirty two patients (80.49%) were CD34+, of them 12 (39.39%) achieved complete remission and 20 (60.61%) did not respond to induction therapy. On the other side 9 patients (19.51) were CD34-; 5 (55.56%) of them achieved complete remission, while 4 (44.44%) did not (Table 3).

Table 3. The Remission status of patients distributed according to the flow cytometric expression of markers

Maker	Remission		Total	P value
	Complete No (%)	Not No (%)		
CD123⁺	8 (29.63%)	19 (70.37%)	27	0.035
CD123⁻	9 (64.29%)	5 (35.71%)	14	
CD34⁺	12 (36.36)	20 (63.64)	33	0.27
CD34⁻	5 (55.56)	4 (44.44)	9	
MPO <20%	8 (29.63%)	19 (70.37%)	27	0.057
MPO ≥20%	8 (61.54%)	5 (38.64%)	13	

Regarding myeloperoxidase; a 20% cut-off value was used to separate two prognostic groups. 7 (58.33%) of 12 patients who had MPO expression of more than 20% achieved complete remission and 5 (41.67) did not achieve

complete remission, while 10 (34.48%) of patients who expressed MPO in less than 20% of cases achieved complete remission and 19 (65.52%) did not.

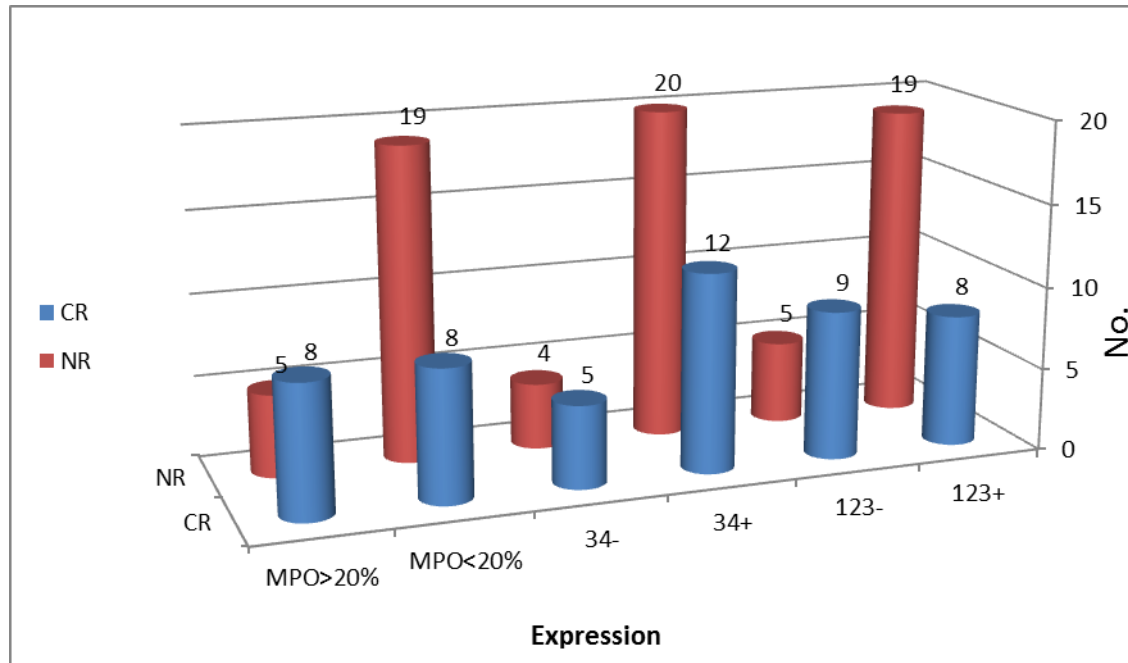


Fig. 1. Remission status of the studied patients according to the expression profile (MPO=myeloperoxidase, CR=complete remission, NR=Non-remission).

Discussion

The mean age of AML patients was 38.73 years and the peak incidence of AML was in the age group 15-24 years, while the least incidence was found in the age group >64 years. Those findings were comparable with the results of survey made by Hussein *et al* ⁽⁴⁾, Al-Husseiny ⁽²²⁾ and Dhahir *et al* ⁽²³⁾. The mean age of patients was markedly lower than that observed in other developed countries where the majority of cases were above 55 years of age ⁽²⁴⁾. This may be attributed to sample size.

The male to female ratio was 1:1.41 which was higher than the average found by previous Iraqis studies ^(4,23).

The mean WBC count, hemoglobin (Hb) concentration and platelet count were comparable to previous Iraqi studies. Leukocytosis ($> 11 \times 10^9/L$) was found in 73% of AML patients which is more than that reported by Al-Husseiny ⁽²²⁾ whereas leukopenia and

normal leukocyte count on presentation were less observed findings than in the other studies ^(4,23).

Regarding FAB subtyping, the findings of the current study were comparable to previous Iraqi studies. FAB M3 was the most common subtype, which was the same reported by most local previous studies. However, Dhahir *et al* ⁽²³⁾ and Alwan *et al* ⁽²⁷⁾ reported that M3 was the third most common factor. This variation may be attributed to differences in sample sizes.

The percentage of CD34⁺ cases was comparable to that found by Oyan *et al* in 2005 ⁽²⁵⁾, but more than that found by Petrovici *et al* ⁽¹⁸⁾ in 2010, whereas CD123 expression was less expressed compared to the study of Muñoz *et al* ⁽²⁶⁾ in 2001. The overall positivity of MPO in the current study was comparable to that found by Buccheri *et al* ⁽¹⁶⁾ and Leong *et al* ⁽²⁷⁾.

In the present study, the rate of induction failure was 58.53% which was high compared to

previous studies from Iraq⁽²⁸⁾. A significant relationship between expression of CD123 and induction failure was found. This finding is comparable to that found by Testa⁽²⁹⁾ in 2002 who stated that elevated expression of CD123 (IL-3R α) in AML is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. CD123 was related to the leukemic nature of stem cells and experiments showed that CD123⁺ cells were competent to establish and maintain leukemic populations *in vivo*⁽²⁸⁾.

The rate of induction failure in CD34⁺ group was higher than that observed in CD34⁻ group. This finding is comparable to the results of previous studies^(19,31) and proves that CD34 expression predicts for poor prognosis in AML patients.

The results of this study were comparable with the results of Matsuo *et al*⁽²⁰⁾ who stated that the percentage of myeloperoxidase-positive blast cells is a strong independent prognostic factor in acute myeloid leukemia, even in the patients with normal karyotype.

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Author Contribution

Research proposal, analysis of results by statistics, collection of samples, patient interview, sample analysis, patients follow up and final printout of article were done by Dr. Faez and the co-author assistant professor Dr. Subh S. Al-Mudallal.

Conflict of Interest

There's no financial or personal relationship with other people that could inappropriately influence this work.

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