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MPO Antigen Negative HLA-DR Negative Acute Myeloid Leukemia: Is it a Separate Clinical Entity?

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ABSTRACT

Background and Purpose: Immunophenotyping is an important tool to assign acute leukemia blast cells to myeloid lineage. The pattern of marker expression in AML is quite heterogeneous even within the same FAB subtype. The whole mark for diagnosis of AML is being MPO positive; the introduction of the MPO antigen (Ag) detection by flow cytometry proved superior to both cytochemistry and electron microscopy. However, lack of MPO Ag in cases which are morphologically and cytochemically proven AML has been reported. The choice of 10% as a cutoff in flow cytometry compared to 3% in cytochemistry may, partly, explain this phenomenon. DR class II MHC is another marker that is supposed to be expressed in most AML cases, except M3. DR negative cases have been previously reported; its impact on prognosis is controversial. Leukemic cells in AML can also express lymphoid-lineage antigens. Studies addressing the prognostic value of immunophenotyping in AML are limited and not conclusive. Up to our best knowledge, there are no studies addressing the prognostic relevance of MPO Ag negative HLA DR negative AML.

The aim of this work is to analyze detailed immunophenotyping in 193 newly diagnosed adult AML patients excluding M3. The prognostic significance of the different markers is verified with special emphasis on MPO Ag negative and HLA-DR negative cases.

Patients and Methods: Excluding M3, one hundred and ninety three newly diagnosed adult AML patients presenting to Medical oncology department of the National Cancer Institute, Cairo University were included in the study. Immunophenotypic analysis was assessed by multicolor flow cytometry. According to MPO Ag% positivity, patients were classified into 3 groups: Group I: MPO Ag negative ≤3%, Group II: MPO Ag weak positive >3<10%, Group III: MPO Ag positive ≥10%. Treatment and follow up: Induction therapy for patients ≤60 years included 2 regimens: Regimen 3 & 7 and HAM regimen. Evaluation of response was done after 2-3 weeks to determine cases in complete remission (CR) or refractory.

Results: Marker expression showed that CD13 and CD33 were the most frequently expressed (89% and 86.5%); 2 cases were CD13 negative and CD33 negative. No significant differences were encountered between MPO Ag weak and MPO Ag positive cases; only CD33 showed near significance association with MPO Ag positive group (p0.06) which attained significance when both positive groups were pooled (p0.02). CD34 and lymphoid marker expression were significantly associated with MPO Ag negative group (p<0.01 and p0.013). The significance was attained even at single marker level namely CD5, CD19 and CD22 and increased when MPO Ag weak positive and MPO Ag positive groups were pooled (p0.008, p0.007 and p0.002). MPO Ag negative DR negative cases (10 patients, 5.2%) had special features: Female predominance (M:F 1:2.33 versus 1.28:1 in the whole group), statistically significant lower peripheral blood blast percent (p0.04) and lower frequency of CD13 and CD34 (p0.004 and p0.025). Out of seventy-eight evaluable patients, CR was achieved in 56. CD14 showed significant association with CR rate (p0.04). No significant association with MPO Ag expression was encountered.

Conclusion: The study emphasizes that >3% should be the cutoff for MPO Ag expression by flow cytometry. A small subset of AML cases (MPO Ag negative DR negative AML patients) apparently showed special characteristics which need collection of a larger number of cases to verify. It seems that the combination rather than a single marker expression would make the difference. Apparently a new era is just starting to stratify AML cases according to immunophenotyping besides the standard FAB categorization.

Key Words: *Immunophenotyping - AML - MPO - HLA DR.*

INTRODUCTION

Acute myeloid leukemia (AML) in adults is a heterogeneous disease, with a variable response to therapy with anticancer agents **[1]**. Using combination chemotherapy protocols,

approximately 60% of patients achieve complete remission (CR) but only a minority remains leukemia free **[2]**.

A variety of clinical and biological parameters have been examined for potential value in predicting treatment response and survival, including age, gender and cytogenetics **[2]**.

Although immunophenotyping is an important tool to assign acute leukemia blast cells to myeloid lineage, its role has been largely confined to differentiate it from ALL and to confirm the diagnosis of M0, M6 and M7 **[3]**. Studies addressing the prognostic value of immunophenotyping in AML are not conclusive. Leukemic myeloblasts express a variety of leucocyte differentiation antigens, which reflect commitment to the myeloid lineage as well as level of differentiation **[4,5]**. These antigenic phenotypes have been proven very useful in the diagnosis of AML, but the prognostic value has remained uncertain and unclear **[5]**. Initial reports suggested a relationship between patterns of myeloid lineage differentiation antigens and patient prognosis but subsequent studies have produced conflicting and inconsistent results **[4,5,6]**. Some markers showed controversial significant prognostic association in more than one study; CD13 **[7,8]**, CD14 **[9,10]**, CD15 **[11]** as well as CD34 which is claimed to be associated with poor clinical outcome in AML **[5,10]**.

Leukemic cells in AML can also express lymphoid-lineage antigens **[12,13]**. The prognostic significance of this phenomenon in AML has also been examined, but with high conflicting claims of poorer or unaltered prognosis **[5,12,14]**.

The pattern of marker expression in AML is quite heterogeneous even within the same FAB subtype **[15]**. This observation has urged trials to develop immunological classifications that could possibly have a prognostic significance **[16,17]**.

The whole mark for diagnosis of AML is being MPO positive; the introduction of the MPO antigen (Ag) detection by flow cytometry proved superior to both cytochemistry and electron microscopy **[18]**. However, lack of MPO Ag in cases which are morphologically and cytochemically proved AML has been reported **[16,17]**. The choice of 10% as a cutoff in flow cytometry compared to 3% in cytochemistry may, partly, explain this phenomenon **[19]**. Another marker that is supposed to be expressed in most AML cases, except M3, is DR class II MHC. DR negative cases have been previously reported; its impact on prognosis is controversial **[6,10,16,20,21]**. Up to our best knowledge, there are no studies addressing the prognostic relevance of MPO Ag negative AML.

In this study, we are analyzing detailed immunophenotyping of 193 newly diagnosed adult AML patients excluding M3. The prognostic significance of the different markers is verified with special emphasis on MPO Ag negative and HLA-DR negative cases.

PATIENTS AND METHODS

Patients:

One hundred and ninety three newly diagnosed adult AML patients presenting to Medical Oncology Department of the National Cancer Institute, Cairo University in the period from 2000 to 2003 were included in the study. Written informed consent was obtained from the patients and the protocol was approved by the Institution Research Board. The age ranged from 18 to 74 years with a median of 31 and a mean of 34.55±31.87 years. They were 108 male and 84 female.

Diagnosis of AML was performed according to standard criteria including clinical, morphological and cytochemical examination. The FAB subtype was determined **[22]**. M3 cases were excluded from the study.

Immunophenotypic Analysis:

Immunophenotypic analysis was performed on mononuclear cells from fresh peripheral blood or bone marrow samples taken at the time of diagnosis. It was assessed by multicolor flow cytometry (Coulter Epics XL, Hialeh). A wide panel of FITC (fluorescin) or PE (phycoerythrin) conjugated monoclonal antibodies (Mo Abs) was used (Table 1). Double and Triple marker labeling was performed, including proper isotype controls.

Detection of Surface Markers by Direct Staining:

The whole blood staining method was performed. In short, 10μl labeled Mo Ab was added to 100μl whole blood, incubated in the dark for

20 minutes then processed by the Q prep system (Coulter Corp, Hialeh, Fl) where immunoprep reagent A for lysing, B as stabilizer and C as fixative were consecutively added. The samples were analyzed on the flow cytomter.

Detection of Intracellular Markers:

Hundred µl of whole blood was lysed using lysis solution (Becton & Dicknson) 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 4°C. Cells were washed, suspended in 500µl PBS and analyzed **[23]**.

Any antigen was considered positive when ≥20% of blast cells were stained above the negative control except for CD34 and CD10 where ≥10% was considered positive. According to MPO Ag% positivity, patients were classified into 3 groups:

1- Group I: MPO negative ≤3%.

2- Group II: MPO weak positive >3<10%.

3- Group III: MPO positive ≥10%.

In an attempt to consider MPO Ag cutoff positivity at a lower percentage (3% instead of 10%), group II and group III were compared to each other and were considered as one group in the statistical analysis thereafter.

Each group was further divided according to DR expression into DR negative (a) and DR positive (b). Fig. (1) shows an AML case MPO Ag +/DR+.

Fig. (2) shows an AML case MPO Ag -/DR.

Treatment and Follow up:

Induction therapy for patients ≤60 years included 2 regimens:

1- Regimen 3 and 7 was given as Duanorubcin 45mg/m^2 or Doxorubicin 40mg/m^2, IV, from day 1 to day 3 and Cytosine arabinoside 100mg/m^2, by continuous infusion, from day 1 to day 7.

2- HAM regimen: By high dose Cytosine arabinoside 1gm/m^2/12 hours from day 1 to day 3 by infusion over 3 hours and Mitoxantrone 12mg/m^2 from day 3 to day 5 by short infusion.

Patients above the age of 60 years (4 cases) received non-anthracyclin containing regimen.

Evaluation of response has been done after 2-3 weeks. Complete remission (CR) was defined as cellular marrow with less than 5% blasts, no circulating blasts, no evidence of extramedullary leukemia and recovery of granulocytes ≥1.5x10^9/L and platelet ≥100x10^9/L.

Patients who attained CR were considered for post remission therapy while those who failed to respond to induction therapy were evaluated as refractory. Post remission therapy was risk adapted: Patients with unfavorable risk and having HLA-identical donor were subjected to high dose therapy and peripheral stem cell transplantation. Those with no available donor or with contraindication for transplantation as well as patients with favorable prognosis were treated with 3 more cycles of HAM regimen and then kept under follow up. DFS and OS were evaluated for all patients in addition; OS was evaluated for the three MPO groups.

Supportive Care:

Blood components transfusion was given to keep the hemoglobin level at 8gm/dl or higher. Theraputic platelet transfusion was given to patients with bleeding manifestation and thrombocytopenia. Prophylactic platelet transfusion was given when platelet count <10x10^9/L or at a higher level if patients had complications or planned for invasive procedure.

Evaluation and management of infection was applied according to the rules recommended for infection management in the immunocompromised patients **[24]** and according to the ongoing institutional protocols.

Statistical Analysis:

Statistical package for social sciences (SPSS) version 9 was used. Quantitative variables were summarized using mean and SD, median, minimum and maximum values. Qualitative data were summarized using frequencies and percentage.

The relation between quantitative variables was tested by Spearman Correlation. Chi or Fisher's exact tests were used whenever appropriate to test the association between the different qualitative variables. Differences were considered significant at a *p* value of ≤0.05 and highly significant at a *p* value of ≤0.01 **[25]**.

RESULTS

The study included 193 newly diagnosed adult AML cases, (excluding M3). FAB classification was available for 105 cases. M1 was the predominant FAB subtype (p0.02). The results are summarized in (Table 2). Peripheral blood blast median was 65% with a range of 20-96% and a mean of 53.74±31.79%.

Marker Expression:

CD13 and CD33 were the most frequently expressed; 2 cases were CD13 negative, CD33 negative; one was M1 (MPO Ag negative, DR negative and CD5 positive) and the other was M5 (MPO Ag positive, DR negative, CD14 negative, CD2 and CD7 positive).

CD14 was significantly associated with M4 and M5 (p<0.01) being expressed in 61.5% and 46% respectively vs. 0% in M0 and M7, 7.3% in M2 and 7.4% in M2.

MPO Ag expression was 61.1% (118/193 cases). According to MPO Ag % positivity cases were divided into 3 groups:

- Group I (MPO Ag negative ≤3%): 47 cases.
- Group II (MPO Ag weak positive >3% and <10%): 28 cases.
- Group III (MPO Ag positive ≥10%): 118 cases.

With regards to myeloid markers expression in context of MPO Ag expression, only CD33 showed near significant association with MPO Ag positive group (p0.06), which attained significance when MPO Ag weak positive and MPO Ag positive groups were, pooled (p0.02).

CD34 showed statistically significant association with MPO Ag negative group (p<0.01). The significance increased when MPO antigen weak positive and MPO Ag positive groups were pooled (p0.005).

Lymphoid marker expression was significantly associated with MPO Ag negative group (p0.013). The significance was attained even at single marker level namely CD5, CD19 and CD22 (p0.03, 0.03 and 0.009 respectively). The significance increased when the MPO Ag weak positive and MPO Ag positive groups were pooled (p0.008, 0.007 and 0.002 respectively).

HLADR Status:

Each of the 3 MPO groups was further divided according to the DR status into DR negative (a) and DR positive (b).

Group I: 47 cases including 10 DR negative (Ia) and 37 DR positive (Ib). Group Ia showed female predominance (M: F 1:2.33), though not statistically significant (p0.08), statistically lower peripheral blood blast percent (p<0.04), lower frequency of CD13 and CD34 (p0.004 and 0.025 respectively). The 2 CD5 positive cases lied in this group (p0.006). Table (3) shows marker expression in the 10 MPO Ag negative DR negative AML cases.

Group II: 28 cases including 7 DR negative (IIa) and 21 DR positive (IIb). None of the tested markers showed any significant association with either group.

Group III: 118 cases, including 35 DR negative (IIIa) and 83 DR positive (IIIb). Group IIIa showed significantly lower frequency of CD13 expression (p<0.01).

Clinical Outcome:

Seventy-eight patients were evaluable while 31 cases showed early death during induction and were considered non-evaluable. The main cause of death was infection, bleeding and organ failure.

Complete remission (CR) was achieved in 56 patients (71.1%). Out of the 56 patients, 6 were subjected to peripheral blood stem cell transplantation as post remission therapy. No significant association was encountered between CR on one hand and age, sex, TLC, or surface markers expression on the other hand except for CD14. CR in CD14 negative cases was 76.5% vs. 50% in CD14 positive AML cases (p0.04) table (4). When cases were stratified according to MPO antigen status, CD14 retained its significance only for the MPO antigen positive group (p0.05). With regard to MPO, no significant impact was encountered but there was grading of the CR rate in the three groups being 59% in MPO Ag –ve, 75% in MPO Ag weak and 83.3% in MPO Ag +ve.

DFS was 44% for all patients with a median observation of 12 and range of 1-33 months. OS was 32% (Fig. 3). OS was 24.2% versus 35.6% in MPO Ag negative group and MPO Ag positive patients respectively (Fig. 4).

Table (1): Panel of monoclonal antibodies (mAbs).

Monoclonal Ab	Clone	Source
Myeloid Markers:		
CD13	My7 - PE	Coulter Hialeah, FL
CD14	RmO52 PE	Coulter Hialeah, FL
CD33	M9 - PE	Coulter Hialeah, FL
CD41	P2 - FITC	Coulter Hialeah, FL
Glycophorin A	11E4B.7.6 (KC16)	Coulter Hialeah, FL
Myeloperoxidase	MPO7 FITC	DAKO
Lymphatic Markers:		
B Lineage:		
CD19	BL6 - FITC	Immunotech Marseille, France
CD22	Sd10 PE	Immunotech Marseille, France
T Lineage:		
CD1	BL6	Coulter Hialeah, FL
CD2	39C1.5 FITC	Coulter Hialeah, FL
CD3	UCHT1 FITC	Coulter Hialeah, FL
CD4	13B8.2 - FITC	Immunotech Marseille, France
CD5	BL1A - PE	Coulter Hialeah, FL
CD7	3A FITC	Coulter Hialeah, FL
CD8	B9.11 - PE	Immunotech Marseille, France
NK:		
CD16	3G8 FITC	Coulter Hialeah, FL
CD56	N901 (NKH-1) PE	Coulter Hialeah, FL
Others:		
CD45	Immu 19.2 - FITC	Coulter Hialeah, FL
HLA-Dr	B8.12.2 FITC	Immunotech Marseille, France
CD10	d5 FITC	Coulter Hialeah, FL
CD34	581	Immunotech Marseille, France
Isotypic Controls:		
IgG1 (Mouse)	FITC	DAKO/COULTER/DIACLONE
IgG1 (Mouse)	PE	DAKO/COULTER/DIACLONE
IgG2a (Mouse)	FITC	DAKO/COULTER/DIACLONE
IgG2a (Mouse)	PE	DAKO/COULTER/DIACLONE

Table (2): Characterization of 193 AML cases according to MPO antigen percent expression.

Parameters	AML cases	Group I (MPO Ag -ve ≤3%)	Group II (MPO Ag weak +ve>3 %< 10%)	Group III (MPO Ag +ve ≥10%)
Age: (years)*	31 (18-74)	26 (18-70)	38 (18-74)	30 (18-70)
M:F	1.28:1	1.19:1	2.5:1	1.14:1
TLC:X109/L*	36 (0.54-236)	35 (0.54-194)	25.6(2.4-78)	36.9(0.6-236)
FAB:	(105 cases)	(29 cases)	(14 cases)	(62 cases)
M0	2 (1.9%)	2 (6.9%)	0	0
M1	47 (44.8%)	14 (48.3%)	4 (28.6%)	29 (46%)
M2	29 (27.6%)	5 (17.2%)	5 (35.7%)	19 (30%)
M4	13 (12.4%)	4 (13.8%)	3 (21.4%)	6 (9.7%)
M5	13 (12.4%)	4 (13.8%)	1 (7.1%)	8 (12.9%)
M7	1 (1%)	0	1 (7.1%)	0
Marker expression:	193 cases	47 cases	28 cases	118 cases
Myeloid lineage:				
CD13	172 (89%)	41 (87%)	24 (85.7%)	107 (90.7%)
CD33	166 (86.5%)	36 (76.6%)	24 (85.7%)	107 (90.7%)
CD14	42 (21.8%)	8 (17%)	6 (21.4%)	28 (23.7%)
CD41	1 (0.5%)	0	1 (3.5%)	0
Lymphoid markers:	73 (37.3%)	27 (55.3%)	8 (32.1%)	38 (31.4%)
CD19	10 (5.2%)	6 (12.8%)	1 (3.6%)	3 (2.6%)
CD22**	3 (1.6%)	3 (6.4%)	0	0
CD24	2 (1%)	1 (2.1%)	0	1 (1.2%)
CD2	12 (6.2%)	2 (4.3%)	4 (14.3%)	6 (5.1%)
CD5	2 (1%)	2 (4.8%)	0	0
CD7	22 (11.4%)	7 (14.9%)	3 (10.7%)	12 (10.3%)
CD4	3 (1.6%)	1 (2.1%)	0	2 (2.4%)
CD16(NK)	9 (4.7%)	2 (4.5%)	2 (7.1%)	5 (4.3%)
CD56(NK)	19 (9.8%)	6 (13%)	1 (3.6%)	12 (10.2%)
Other markers:				
HLADR	141 (73.1%)	37 (78.7%)	21 (75%)	83 (70.3%)
CD34	50 (26.7%)	18 (43.9%)	8 (28.6%)	24 (20.3%)
CD10	1 (0.5%)	0	0	1 (1.2%)

* Median (range). ** Cytoplasmic CD22.

Table (3): Marker expression in MPO antigen negative DR negative AML cases.

Cases	FAB	CD13	CD33	CD14	CD34	CD19	CD22	CD2	CD5	CD7	CD16	CD56
Case 1	M0	–	+	–	+	–	–	–	+	–	–	–
Case 2	M4	–	+	+	–	+	–	–	–	–	–	–
Case 3	M1	+	–	–	–	–	–	–	–	+	–	–
Case 4	M2	+	+	+	–	–	–	–	–	–	+	–
Case 5	M1	+	+	–	–	+	+	–	–	–	–	–
Case 6	M0	+	+	–	–	–	–	–	–	+	–	–
Case 7*	M1	–	–	–	–	–	–	–	+	–	–	–
Case 8	M1	+	+	–	–	–	–	–	–	–	–	–
Case 9	M1	+	+	–	–	–	–	–	–	–	–	–
Case 10	M2	–	+	–	–	–	–	–	–	–	–	–

–: Negative.
+: Positive.
*: Sudan black B positive.

Table (4): CR rate in relation to surface marker expression in 78 evaluable AML cases.

Surface markers	Positive cases out of 78 evaluable patients		CR rat		*p*-value*
	Number	Percent	Number	Percent	
CD13	70	89.7	50	71.4	0.8
CD33	61	78.2	45	73.8	0.46
CD14	14	17.9	7	50	0.045
HLA-DR	54	69.2	39	72.2	0.9
CD34	28	35.9	19	67.8	0.54
CD2	5	6.4	4	80	0.5
CD7	10	12.8	8	80	0.6
CD16	3	3.8	2	66.7	0.8

* Significance $p \leq .0.05$.

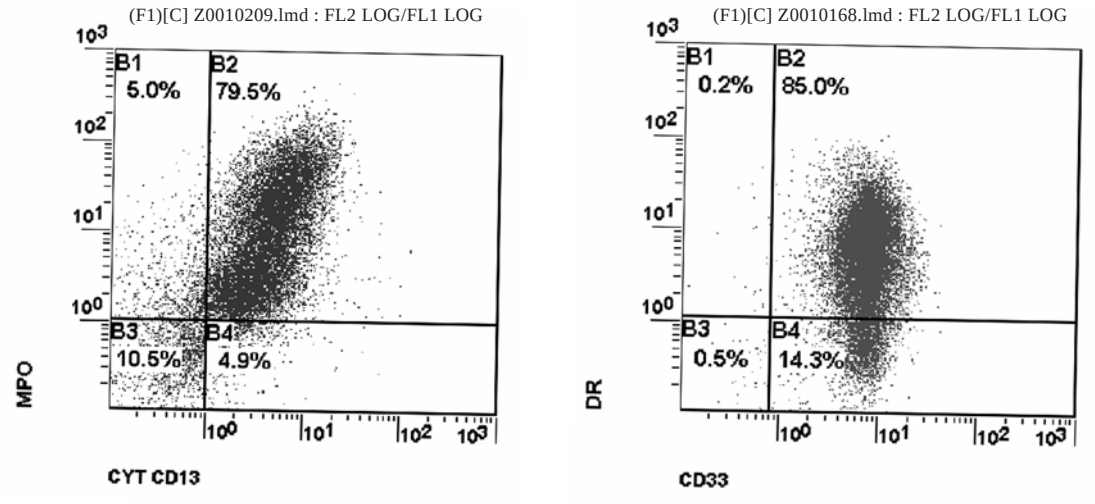

Fig. (1): An AML case MPO Ag +/DR+.

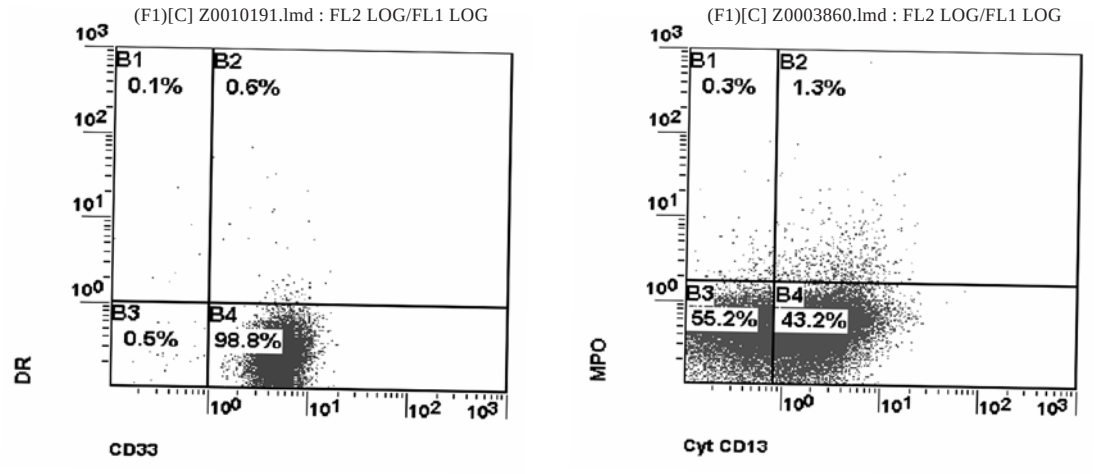

Fig. (2): An AML case MPO Ag -/DR-.

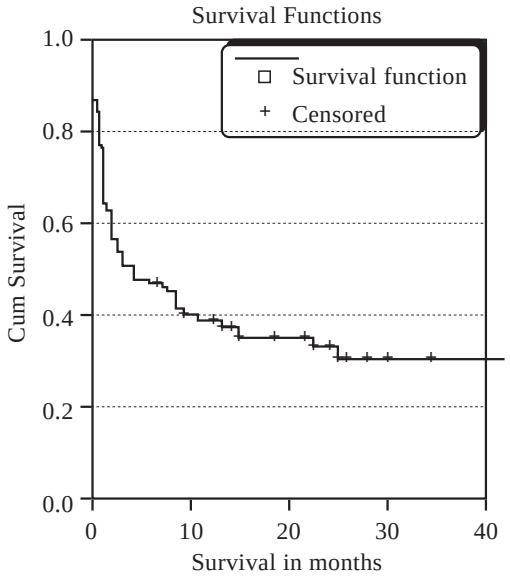

Fig. (3): Overall survival (O.S) in AML cases.

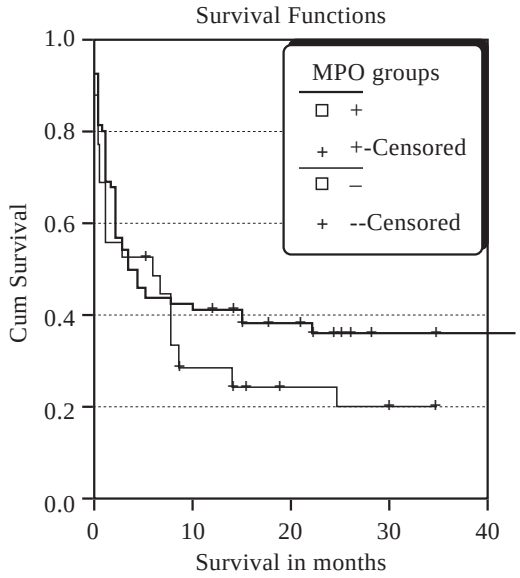

Fig. (4): Overall survival (O.S) in MPO Ag –ve vs. MPO Ag +ve cases.

DISCUSSION

In this work we have studied 193 newly diagnosed AML cases. Their age ranged from 18-74 with a median of 31 years. This is in agreement with previous Egyptian studies that reported a similar median age **[26,27]** but it is much lower than what is reported in Western series with a median of 64 years **[28]**. The male: female ratio in this study was 1.28:1 which is comparable to previous Egyptian reports **[26,27]** and to Western data **[17]**. TLC median in this study was $36x10^9$/L which is higher than corresponding figures in Western series being $15x10^9$/L in the largest series available comprising 909 patients **[17]**. In the present study, M1 was significantly the predominant FAB subtype being 44.8% of our cases. This is comparable to results reported by a previous Egyptian study which was 47.1% **[27]** compared to about 27% in the largest Western series so far reported **[17]** denoting a relatively more immature nature of our cases. The incidence of M4 and M5 in the present study is comparable to Casasnovas' report **[17]**.

The present study emphasizes the marked heterogeneity of AML immunophenoytypes reported in the literature **[1]**. As previously reported, CD13 and CD33 were the most frequently encountered. CD13, CD33 and CD117 combined were claimed to be superior in the

diagnosis of AML **[17]** than the unique detection of MPO antigen **[18]**. Lack of expression of both CD13 and CD33 was encountered in 2 cases of our series. CD13 and CD33 negative AML cases have been previously reported and were viewed as possible examples of extreme asynchrony in sequence of morphologic and immunologic maturation or abnormal epitope expression in leukemic cell molecules **[8]**. CD14 was encountered in 21.8% of our cases; figures in the literature varied between 9% and 35% probably reflecting the relative incidence of M4 and M5 **[5,10,16,17]**. Another factor, however, is the known epitopic variability of the antigen **[5]**. The frequency of CD34 expression in our series was 26.7%. A similar figure was reported **[16]**. However this figure is much lower than that reported by most workers with a frequency of 42-65% **[5,10,17]**. The lymphoid marker expression encountered in our series was 37.3%; the most frequent being CD7 (11.4%) followed by CD2 (6.2%), CD19 (5.2%) CD22 (1.6%) and CD5 (1%); other markers were very low. A comparable figure was reported **[14]**. We have previously reported an incidence of 20% lymphoid marker expression being 23.8% in children and 15.4 in adults **[29]**. The two cases expressing cyt CD22 are considered biphenotypic; they were not excluded on account of their classical morphological and cytochemical patterns; they were both M1. Variable incidence of lymphoid marker expression was reported with figures as low as 15% **[16]** and as high as 44-50% **[5,10,17,30]**. A lower frequency of CD7 (9%) was reported in a large series of 909 studied by Casasnovas et al. **[17]** but higher figures varying from 15 to 32% were reported by others **[5,6,10,16,30]**. Expression of other lymphoid markers were also variable **[5,6, 10,17,30]**.

DR was expressed on 73.1% of our cases. The figures previously reported varied between 65% and 90% **[10]**. Our figures for the various maker expression were nearest to those of Casasnovas et al. **[17]** in their 909 patient series. In this work we categorized our patients according to MPO Ag expression into MPO Ag negative group <3% (29.4%), a group with weak MPO Ag expression of >3 <10% (14.5%) and a MPO Ag positive group with ≥10% MPO Ag +ve blast cells (61.1%). Excluding MPO Ag negative AML has been previously reported **[16]** in 49/325 (15%) of their cases and by Casanovas et al. **[17]** in a special subset of their series (CD13-ve, CD33-ve) to be 39%. The standard cutoff for MPO Ag expression by Flow Cytometry is ≥10%. However, this has been recently criticized and the cutoff of >3% used for cytochemistry was adopted **[19]**.

The cutoff for cytochemistry was determined on the assumption that the bone marrow could, normally, contain up to 3% myeloblasts; any extra blast cell(s) would belong to the leukemic population. When Flow cytometry was used for detection of MPO Ag, this fact was ignored and a cutoff similar to that adopted for other markers e.g. CD10 was automatically applied. In fact, we would go further and suggest that any MPO Ag positive blast cells in the peripheral blood whether detected by cytochemistry or by Flow cytometry should be taken as an indication of the myeloid nature of the leukemia provided that the cell is definitely documented as a blast and not one of the more mature myeloid series namely a promyelocyte or a myelocyte. In all previous reports on MPO Ag negative AML, the 10% cutoff level was used and neither MPO or DR status were considered in the two available trials to establish an immunological classification of AML **[16,17]**.

In this work, Lack of MPO Ag (≤3%) showed significant association with CD34 and lymphoid markers expression while MPO Ag expression showed nearly significant association with CD33 (p0.06) but no association with FAB. This denotes a less differentiated phenotype of the MPO negative AML expressing the stem cell marker (CD34), lacking the myeloid marker (CD33) and having aberrant expression of lymphoid markers; the cell has not yet frankly adopted the myeloid differentiation pathway from the point of view of marker expression even though it is morphologically and cytochemically documented as AML. Taking in consideration that the majority of our cases were M1 which is still early in the pathway of differentiation we may speculate that some of them have not yet acquired the full array of myeloid markers and that the malignant transformation had occurred in a relatively immature cell.

In this work the group with weak MPO Ag expression (>3 <10%) showed no significant association with any other markers, probably on account of its small number. When the two positive groups were pooled together the sig-

nificant associations remained the same and the nearly significant association with CD33 (p0.06) acquired statistical significance (p0.02) which is in harmony with the assumption that the more differentiated the cells, the more myeloid markers they express. It also emphasizes the suggestion that the cutoff should be >3 rather than ≥10.

In this work we have further subdivided our AML cases according to the DR status. The MPO –ve DR –ve group showed significant lower frequency of other markers namely CD13 and CD34 (p=0.004 and 0.002 respectively). Thus we, apparently, have a subgroup lacking many markers denoting relative immaturity. This group showed female predominance though insignificant (p=0.08) against male predominance in the whole series. It also showed significantly lower percentage of peripheral blood blasts (p=0.04) and the 2 CD5 positive cases lied in this group (p=0.006). Thus, in spite of the small number being only 10 cases, this group has a certain pattern, the significance of which awaits studying of a larger number.

Within the MPO Ag +ve group, there was significant association between CD13 and DR which further emphasizes the findings in the MPO Ag –ve group. There was, as well, significant negative association with CD14; this is an expected finding as CD14 +ve monocytic leukemia usually lack MPO. The same applies to the significant positive association between CD14 and DR, where monocytic cells are expected to express class II MHC.

In the present study, CR was attained in 56/78 evaluable cases (71.7%). The corresponding figures in the literature ranged from 60-80% **[17,31,32]**. DFS was 44% at a median observation period of 12 months. Reported DFS at 4 years was 30-40% in patients less than 60 years **[32]**. In the present study, 6 of our patients received post remission allogenic peripheral blood stem cell transplantation (SCT). This was not based on randomization but rather on availability of a matched donor and fulfilling specific eligibility criteria such as age. Comparative analysis in AML patients achieving CR consistently showed markedly reduced relapse rate following allogenic SCT **[33,34]**.

We have studied the impact of various marker expression on CR. CD14 was associated with a lower CR (50%vs.76.6%, p=0.045). When analyzed in context of MPO Ag expression, it retained its significance only in the MPO Ag positive group (p<0.05) while the significance was lost for the MPO Ag –ve group (p=0.329). None of the other markers showed impact on CR including CD34 and CD7. None of the markers studied showed an impact on survival. Previous reports have addressed the prognostic relevance of various markers. CD14 was reported to be associated with low OS **[17,30]** especially when associated with CD7 **[30]**. CD7 is one of the most extensively studied markers with a lot of controversy. In the aforementioned study, it showed an adverse effect on CR (p<0.002), CCR (p<0.001) and OS (p<0.001) which was confirmed by multivariate analysis. The adverse effect has been reported by other workers **[35,36,37]**. On the other hand this was denied by Tien et al. **[13]**. With regard to CD34 expression, the majority of reports documented its adverse effect as an independent prognostic factor **[17,38]** which we failed to detect in this series and in a previous one as well **[39]** Sporadic reports are available for other markers including a high CR rate in CD2+ CD19+ AML cases with superior survival **[14]**, lack of prognostic impact of HLA DR –ve cases, if M3 is excluded **[21]**. The marked controversy in the reports on the prognostic value of different markers has urged the search for an immunological classification of AML that might have a prognostic relevance. Two main proposals for such a classification are available in the literature. In the first **[16]**, the authors concentrated on developing a scoring system for better discrimination between M0 and ALL. The second **[17]** comprised 909 AML cases studied in two stages, 176 as a test series and 733 as a training series to validate the findings obtained from studying the first group. They classified the cases according to the pattern of marker expression into five groups (MA-ME), taking in account the expression of 4 groups of markers namely CD13 or CD33 or CD117, CD7, CD35 or CD36, and CD15. They tested the prognostic impact of other markers in context of these five groups (MA-ME). Specific independent prognostic factors were related to poor overall survival in each of these groups namely CD34+ in MA and MD, CD7+ in MB-non- APL, and CD14 in MD and ME. The study emphasizes the findings obtained in this work that it is the combination rather than a single marker expression which might make the difference, though we used two different markers

namely MPO and DR for categorization of our cases. It seems that a new era is just starting to evaluate the prognostic value of immunophenotyping of AML which was previously confined, mostly, to diagnose M0, M6 and M7.

In Conclusion: The role of immunophenotyping in AML is not, any more, confined to diagnosis. Characterization of specific subsets is required to establish an immunological classification with potential prognostic relevance. In this study, as a first step, we have indicated a potentially distinct subtype namely MPO Ag –ve HLA DR –ve. A large number of this apparently infrequent phenotype is needed to verify its significance.

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