Prognostic Significance of Progenitor Cell Markers CD34/CD38 Expression in Acute Myeloid Leukemia (AML) Egyptian Patients

Naglaa Mostafa[1], Reham A. Rashed[1], Waleed S. Mohamed[2], Hanan E. Shafik[3]

Departments of [1]Clinical Pathology, [2]Cancer Biology, [3]Medical Oncology, National Cancer Institute, Cairo University, Egypt
E-mail: reham_r9@yahoo.com

Abstract: Introduction: The relapse of Acute Myeloid Leukemia (AML) is thought to reflect the failure of current therapies to target leukemia stem cells, typically enriched in the CD34/CD38 cell population. The aim of this study was to determine the prognostic significance of progenitor cell markers CD34/CD38 in AML. **Methods:** Progenitor cell markers CD34/CD38 expression was determined on bone marrow mononuclear cells of 84 newly diagnosed adult AML patients with 18 age and sex matched controls, using CD38FITC/CD34PE panel of monoclonal antibodies and analyzed by Flowcytometry technique. **Results:** Expression of CD34 and CD38 cell markers was detected in 79.8% and 85.7% of AML patients respectively, and there was a highly significant difference of CD 34 expression among cases and controls ($p \leq 0.001$). No significant correlation was found between both markers and any of the hematological findings, cytogenetic and FLT3 mutation except with peripheral blood blasts (p=0.05 and 0.005, respectively) and FAB subtypes for CD34 (p=0.006). A significant correlation was found between various CD34/CD38 groups and total leucocytic count, hemoglobin, peripheral blood blasts, and FAB subtypes (p=0.05, 0.047, 0.035 and 0.002 respectively). Also, there was no significant association between both markers expressed separately or in combination with response rate, overall survival and progression free survival. **Conclusion:** Both progenitor cell markers CD34/CD38 expression might be used as susceptible markers providing important clues for future studies in the early detection of resistant AML cases.

[Naglaa Mostafa, Reham A. Rashed, Waleed S. Mohamed, Hanan E. Shafik. **Prognostic Significance of Progenitor Cell Markers CD34/CD38 Expression in Acute Myeloid Leukemia (AML) Egyptian Patients.** *Cancer Biology* 2016;6(2):1-10]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online). http://www.cancerbio.net. x. doi:10.7537/marscbj06021601.

Key words: Prognostic significance- CD34- CD38- AML

1. Introduction

Acute myeloid leukemia (AML), the most frequent aggressive hematological malignancy in adults characterized by an accumulation and differentiation arrest of myeloid progenitor cells in the bone marrow and blood that requires immediate treatment [1]. Although most patients with acute myeloid leukemia (AML) achieve complete remission (CR) after standard induction chemotherapy, the majority subsequently relapse and die of the disease. A leukemia stem cell (LSC) paradigm may explain this failure of CR to reliably translate into cure [2]. Leukemic stem cells (LSCs) seem to host biological properties that render them resistant to chemotherapy and thus might be responsible for minimal residual disease (MRD). So targeting minimal residual disease (MRD) to prevent relapse is one of the major challenges in treatment of acute leukemias [3]. Various methods with different sensitivities, including flowcytometry (FC), chimerism, cytogenetics, and molecular analysis, have been used [4]. FC has become the gold standard for evaluating MRD in patients with acute myeloid leukemia. However, up to 20% of patients with AML lack appropriate markers for MRD follow-up at diagnosis, and changes in the original immunophenotype might occur in relapse. LSCs, cell compartments could be defined by immunophenotyping [5, 6]. The first LSC compartment that was described had the CD34+CD38- immunophenotype, it seemed previously to be the most robust compartment in CD34 positive (CD34+) patients, since it was found to be the predominant compartment containing leukemia initiating cells in less immune compromised mouse models [7, 8]. But recent studies have shown that LSCs may reside not only in CD34+CD38-, but also in CD34+CD38+ and CD34- compartments [9]. CD34 is expressed on the surface of immature hematopoietic normal progenitor cells that compromise 1-2% of the cells, it is not lineage restricted and thus not useful for distinguishing AML from ALL [10]. In addition, CD34 is involved in cellular adhesion and mediates resistance to apoptosis [11]. CD34 AML blast cells are even more resistant to programmed cell death with increased percentages of CD34 cells [12]. CD38 is mostly expressed on the surface of immature cells and different lineages of hematopoietic activated cells like lymphocytes and myelocytes [10]. Moreover, CD38 is

supposed to mediate signaling pathways that result in cell proliferation, regulation of apoptosis and differentiation. It also serves as a cell adhesion molecule [10].Therefore, the aim of the present study was to evaluate and investigate the expression of progenitor cell markers CD34, CD38 on AML blasts at initial diagnosis, especially the expression characteristics of each single marker and in combination to enlighten their diagnostic and prognostic relevance.

2. Materials and Methods

2.1. Research design and setting

This study was carried out on 84 newly diagnosed AML patients who presented to the Medical Oncology Department, National Cancer Institute (NCI); Cairo University over a period of 24 months with 18 age and sex matched controls. A Written informed consent was obtained from each patient before starting the data collection. It was approved by the Institutional Review board (IRB) ethical committee of the NCI which follows the rules of Helsinki IRB. For the sake of patient's privacy, they were given code numbers.

2.2. Collection of the sample

One to two ml of bone marrow samples were collected on EDTA from each patient at diagnosis and its controls (obtained from bone marrow transplant donors). Diagnosis was established after proper clinical examination, Leishmans' stained blood and BM smears for morphological assessment supplemented with cytochemical stains (Peroxidase (MPO) or Sudan Black Stain (S.B.B), Estrases, Acid Phosphatase and PAS when indicated).

2.3. Cytogenetic analysis (Conventional karyotyping)

Cytogenetic examination that involves the examination of spontaneously dividing cell populations by blocking cell division at metaphase stage with an inhibitor of spindle formation (Colcemid), this is followed by fixative then hypotonic wash and slide making and staining with Giemsa using trypsin to induce G banding. Analysis of available metaphases were counted and analyzed under microscope and 20 metaphases were captured, analyzed and karyotyped using image system cytovision/genus application software versus 4.02. FISH as a complementary tool to conventional cytogenetic when indicated and FLT3/ITD mutation detection (on routine basis), all the cases met the AML diagnosis standards [13].

2.4. Cell preparation and flow cytometric analysis

Flow cytometric immunophenotyping of blast cells was performed on multicolor flow cytometry (Navios), Navios software applied for analysis using whole blood lysis method. A panel of mouse monoclonal antibodies directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or tandem Cy5-PE (PC5) were used. These monoclonal antibodies included myeloid markers (CD13, CD33, CD117, CD14, CD15 and myeloperoxidase), lymphoid markers (CD10, CD19, CD20, CD5, CD2, CD7 and CD3) on routine basis as well as PE labeled anti CD34 and FITC labeled anti CD38, (Dako Company).

2.5. Direct staining of cell surface antigens

Incubate 2×10^6 cells in 100µl of sample with 10µl of a fluorescent monoclonal antibody specific for CD38 and CD34 (FITC and PE respectively) for 30-45 minutes at 4°C. After incubation, add 1-2 ml of lysing solution, incubate for 5-10 minutes and then centrifuge at 3000 rpm for 3 minutes. Wash with PBS twice. Resuspend the pellet in 0.5 ml of buffer. The samples were analysed by a multicolor flow cytometry (Navios). Debris was excluded from the analysis. An appropriate isotype control IgG1 was used in all cases to assess background fluorescence intensity. The leukemic cell population was identified by gating the typical formation in the forward/side scatter projection, with residual lymphocytes. Results were expressed as the percentage of cells showing positive expression, 5000-10000 cells in the gate were analyzed. If the percentage of positive events was >20%, the case was considered as positive for that surface marker as well as progenitor cell markers**,** except for CD34 and intracellular MPO where expression ≥10% was considered positive. All patients received induction chemotherapy using 3&7 regimen (Cytarabin 100mg/m2 continuous infusion for 7 days and Adriamycin 20mg/m2 for 3 days). The patients were assessed for response on day 28. Patients who reached CR were consolidated by 2 cycles of high dose Cytarabin and metoxantrone (HAM). Median duration of follow up was 24 months.

2.6. Statistical analysis

Data management and analysis was performed using SPSS, version 20. Categorical data were summarized as percentages; numerical data were summarized using means and standard deviation or medians and range. Relation of CD34 and CD38 with other variables was assessed using Chi-square test. Overall survival (OS) was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Progression free survival (PFS) was defined as the time from starting therapy until documented progression or death. For patients without disease progression (DP) at the time of analysis, the date of last follow-up was considered right-censored. OS and PFS were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival

curves. All tests of hypotheses were conducted at the alpha of 0.05 level, with a 95% confidence interval.

3. Results

Successful karyotyping and laboratory findings were done to 84 patients; FLT3 assessment was available for 49 patients only. The general characteristics, hematological findings, FAB classification, karyotyping and FLT3 mutation among 84 adult AML cases are listed in Table (1). CD34 and CD38 progenitor markers were studied in the 84 AML patients and 18 healthy controls, we defined our patient subgroups as positive and negative for each marker. If the percentage of positive events was >20%, the case was considered positive for CD38**,** while for CD34, only ≥10% was considered positive**.** As regards the expression of both markers studied among cases and controls, none of the studied controls expressed CD34 (0%), while all of them (100%) were CD38 positive. No statistically significant difference of CD38 expression was found between cases and controls, where it was 85.5% and 100% respectively (p=0.081), while positive CD34 expression showed a highly statistically significant difference, (79.8% and 0%) (p<0.001). Table (2) & Table (3) represent the hematological parameters, FAB classification, cytogenetic analysis and FLT3 mutation in relation to positive and negative expression of CD34 and CD38 markers. There was no statistically significant difference between positive and negative expressions for both markers except with peripheral blood blast count, where frequency of patients with PB blasts <50% were significantly higher in negative and positive CD34 expression compared to those having PB blasts ≥50% (P=0.05), and frequency of patients with PB blasts <50% were significantly higher in negative CD38 expression compared to those having PB blasts ≥50% (P=0.005) and with the different subtypes of FAB classification regarding CD34 expression where those negative for CD34 were represented in M1 and M3 and those positive for CD34 were represented in M1 and M2 (p=0.006). Cases and controls were divided into 4 different groups in relation to the combined CD34/CD38 expression. All of the controls were CD34-ve/CD38+ve group (100%), while CD34+ve/CD38+ve group was the most frequent in cases (70.2%), with a high statistical significant difference reached (P=0.005) Table (4). Table (5) represents the relation of CD34/CD38 groups with different hematological finding, FAB classification, karyotyping and FLT3 mutation. A highly statistical significant correlation was found between all the groups and low TLC count (< 100) (P=0.05) , low HB level (<8) (P=0.047), and low PB blast count (<50) (P=0.035), and finally a high statistical difference with the different subtypes of FAB classification where the 1st group was represented in M1-M3 , 2nd group was represented in M2, 3rd group was represented in M1-M3 and finally the 4th group was represented in M1-M4 (p=0.002). Concerning the complete response rate (CR), successful follow up was achieved for (67/84, 80%) of patients while (17/84, 20%) died during the study. As regards CD34 and CD38 expression with the response rate, among those with positive CD34 expression (47/57, 82.5%) achieved CR compared to (10/57, 17.5%) that failed to achieve CR. In those with negative CD34 (9/10, 90%) achieved CR compared to (1/10, 10%) showing no significant difference among positive and negative CD34 expression in relation to response rate (P=0.47). While for CD38 expression, patients with positive expression (47/56, 83.9%), achieved CR compared to the (9/56, 16.1%) who failed to achieve CR. On the other hand, the patients who showed negative expression (10/11, 90.9%) achieved CR compared to those (1/11, 9.1%) who failed to achieve CR showing no statistical significance difference between positive and negative CD38 expression in relation to response rate (P=0.48). Considering CD34/CD38 groups, no statistical significant difference was found between the 4 different groups as regards response rate (87.5%, 87.5%, 83.3%, and 100% respectively) (P=0.87) Table (6). In our study, the median overall survival (OS) for all patients was 4.67 months (95% CI: 2.06-7.28) and the median progression free survival (PFS) was found to be 19.1ms (95% CI 5.85-32.36). There was no statistically significant difference in OS and PFS as regards CD34 and CD38 positive and negative expressions (P=0.74, 0.98, 0.59 and 0.79 respectively). As regards CD34/CD38 groups; CD34+ve/CD38-ve group had a median OS of 9.77ms (95%CI: 0-21.58) compared to 3.58ms (95% CI: 1.06-6.11) for other groups, with no statistical significant difference (P=0.37), while median PFS was not reached compared to 19.11ms (95% CI: 5.71-32.52) for other groups (P=0.25). Finally, there was no statistical significant difference between the FLT3 wild and mutant patients in mortality rate (59% vs 91.6%, p= 0.08). On the other hand, the progression free survival (relapse rate) showed a statistical significant difference between the FLT3 wild and mutant patients (8.1% vs 33.3%, p <0.001).

Table 1. CD34 and CD38 expression among Cases and Controls

		Cases versus control		p-value
		Control; n=18	Cases; n=84	
CD34	Negative	18 (100%)	17 (20.2%)	<0.001*
	Positive	0 (0%)	67 (79.8%)	
CD38	Negative	0 (0%)	12 (14.3%)	0.081
	Positive	18 (100%)	72 (85.7%)	

*****Significant

Table 2. Clinical and Hematological Characteristics of 84 adult AML cases

Parameter; Mean ± SD		Cases (%); n=84
Age (Years); 32.9±1.3	<60	82 (97.6%)
	≥60	2 (2.4%)
Sex	Female	33 (39.3%)
	Male	51 (60.7%)
WBCs (x 10^9/L) ; 53.7 ± 6.8	< 100	69 (82.1%)
	≥ 100	15 (17.9%)
HB (g/DL); 7.5 ± 0.2	< 8	52 (61.9%)
	≥ 8	32 (38.1%)
Platelets (x 10^9/L); 63.6 ± 11.1	< 100	71 (84.5%)
	≥ 100	13 (15.5%)
PB blast count; 45.1% ± 3.3	<50	47 (56%)
	≥50	37 (44%)
BM blast count; 53.8% ± 3.1	<50	47 (56%)
	≥50	37 (44%)
FAB Classification	M0	2 (2.4%)
	M1	30 (35.7%)
	M2	30 (35.7%)
	M3	6 (7.2%)
	M4	15 (17.9%)
	M7	1 (1.2%)
Cytogenetic and molecular markers	Normal karyotype intermediate risk	53/84 (63.1%)
	t (8; 21)	13/84 (15.4%))
	t (15; 17)	5/84 (5.9%)
	inv (16)	7/84 (8.3%)
	T (9;22)	1/84 (1.2%)
	Others	53/84 (63.1%)
FLT3	Wild	37/49 (75.5%)
	Mutant	12/49 (24.5%)

n= number, WBCs (White blood cells), HB (Hemoglobin), PB (Peripheral blood), BM (Bone marrow), FAB (French American British), FLT3 (fms-related tyrosine kinase 3)

Table 3. Different Clinical and Hematological Parameters in Relation to CD34 and CD38 expression

		CD34		p-value	CD38		p-value
		Negative n=17	Positive n=67		Negative n=12	Positive n=72	
Age (Years)	<60 ≥60	17 (100%) 0 (0.0%)	65 (97%) 2(3%)	0.63	12(100.0%) 0(0.0%)	70 (97.2%) 2(2.8%)	0.73
WBCs	<100 ≥100	15(88.2%) 2(11.8%)	54(80.6%) 13(19.4%)	0.4	11(91.7%) 1(8.3%)	58(80.6%) 14(19.4%)	0.31
HB	<8 ≥8	9(52.9%) 8(47.1%)	43(64.2%) 24(35.8%)	0.28	8(66.7%) 4(33.3%)	44(61.2%) 28(39.8%)	0.47
Platelets	<100 ≥100	16(94.1%) 1(5.9%)	55(82.1%) 12(17.9%)	0.2	11(91.7%) 1(8.3%)	60(83.3%) 12(16.7%)	0.40
PB blast %	<50 ≥50	13(76.5%) 4(23.5%)	34(50.7%) 33(49.3%)	0.05*	11(91.7%) 1(8.3%)	36(50%) 36(50%)	0.005*
BM blast %	<50 ≥50	8(47.1%) 9(52.9%)	26(38.8%) 41(61.2%)	0.36	6(50.0%) 6(50.0%)	28(38.9%) 44(61.1%)	0.528
FAB	M0 M1 M2 M3 M4 M7	1(5.9%) 6(35.3%) 2(11.8%) 4(23.5%) 3(17.6%) 1(5.9%)	1(1.5%) 24(35.8%) 28(41.8%) 2(3.0%) 12(17.9%) 0(0.0%)	0.006*	1(8.3%) 4(33.3%) 4(33.3%) 0(0.0%) 3(25.0%) 0(0.0%)	1(1.4%) 26(36.1%) 26(36.1%) 6(8.3%) 12(16.7%) 1(1.4%)	0.099
Cytogenetic	Favorable risk Intermediate risk	4(25.0%) 12(75.0%)	21(33.3%) 42(66.7%)	0.38	4(36.4%) 7(63.6%)	21(30.9%) 47(70.1%)	0.242
FLT3	Wild Mutant	8(67.0%) 4(33.0%)	27(73.0%) 10(27.0%)	0.4	3(75.0%) 1(25.0%)	34(75.6%) 11(24.4%)	0.44

*Significant

Table 4. Cases and Controls Frequencies among different CD34/CD38 groups

CD34/CD38 Groups	Cases versus control		p-value
	Control N=18	Cases N=84	
CD34-ve/CD38+ve	18(100.0%)	13(15.5%)	<0.005*
CD34+ve/CD38-ve	0(0.0%)	8(9.5%)	
CD34+ve/CD38+ve	0(0.0%)	59(70.2%)	
CD34-ve/CD38-ve	0(0.0%)	4(4.8%)	

*Significant

Table 5. CD34/CD38 Groups in Relation to Hematological and Laboratory findings in 84 AML cases.

		CD34/CD38 Groups				p-value
		CD34-Ve /CD38+ve (1st group) n=13	CD34+ve/CD38-Ve(2nd group) n=8	CD34+ve/CD38+ve (3rd group) n=59	CD34-ve/CD38-ve (4th group)n=4	
Age	<60 ≥60	13(100.0%) 0(0.0%)	8(100.0%) 0(0.0%)	57(96.6%) 2(3.4%)	4(100.0%) 0(0.0%)	0.495
TLC	<100 ≥100	12(92.3%) 1(7.7%)	8(100.0%) 0(0.0%)	46(78%) 13(22%)	3(75.0%) 1(25.0%)	0.05*
HB	<8 ≥8	6(46.2%) 7(53.8%)	5(62.5%) 3(37.5%)	38(64.4%) 21(35.6%)	3(75.0%) 1(25.0%)	0.047*
Platelets	<100 ≥100	12(92.3%) 1(7.7%)	7(87.5%) 1(12.5%)	48(81.4%) 11(18.6%)	4(100.0%) 0(0.0%)	0.127
PB blast %	<50 ≥50	9(69.2%) 4(30.8%)	7(87.5%) 1(12.5%)	27(45.8%) 32(54.2%)	4(100.0%) 0(0.0%)	0.035*
BM blast %	<50 ≥50	7(53.8%) 6(46.2%)	5(62.5%) 3(37.5%)	21(35.6%) 38(64.4%)	1(25.0%) 3(75.0%)	0.279
FAB	M0 M1 M2 M3 M4 M7	1(7.7%) 4(30.8%) 2(15.4%) 4(30.8%) 1(7.7%) 1(7.7%)	1 (12.5) 2 (25%) 4 (50%) 0(0.0%) 1 (12.5) 0(0.0%)	0(0.0%) 22(37.3%) 24 (40.7%) 2 (3.4%) 11 (18.6%) 0(0.0%)	0(0.0%) 2 (50%) 0(0.0%) 0(0.0%) 2 (50%) 0(0.0%)	0.002*
Cytogenetic	Favorable risk Intermediate risk	4(30.8%) 9(69.2%)	4(50.0%) 4(50.0%)	16(29.6%) 38(70.4%)	0(0.0%) 4(100.0%)	0.247
FLT3	Wild Mutant	8(88.9%) 1(11.1%)	0(0.0%) 0(0.0%)	26(72.2%) 10(27.8%)	3(75.0%) 1(25.0%)	0.44

*Significant

Table 6. ***CD34, CD38 and CD34/CD38 Groups Expression in relation to Response rate***

Parameter		Response rate		p-value
n=67		No response (NR)	Complete remission (CR)	
CD 34	Negative N=10 Positive N=57	1(10.0%) 10(17.5%)	9(90.0%) 47(82.5%)	0.47
CD38	Negative N=11 Positive N=56	1(9.1%) 9(16.1%)	10(90.9%) 47(83.9%)	0.48
CD34-ve/CD38+ve N=8 CD34+ve/CD38-ve N=8 CD34+ve/CD38+ve N=48 CD34-ve/CD38-ve N=3		1(12.5%) 1(12.5%) 8(17.6%) 0(0.0%)	7(87.5%) 7(87.5%) 40(83.3%) 3(100.0%)	0.87

4. Discussion

AML is regarded to originate in the hematopoietic stem cell compartment. The lack of durable response in a high percentage of AML patients suggests that current treatments do not effectively target LSCs, and one of the major challenges in the design of new therapies to eradicate LSCs is to achieve high therapeutic specificity [14].
Flow Cytometric analysis of blast cells improves both accuracy and reproducibility of the FAB classification and is considerable practically useful for the detection of MRD by monitoring AML cases in remission [10]. The purpose of the current study was to investigate the expression of progenitor cell markers CD34, CD38 on AML blasts at initial diagnosis, especially the expression characteristics of each single marker separately or in combination to evaluate their diagnostic and prognostic relevance in AML patients and to establish a relationship between them with the response to chemotherapy and clinical outcome. In our study, none of the healthy controls showed positive CD34 expression, our results is near to what was found by Wen et al., [15] who showed in their analysis that AML had higher CD34+ and CD34+CD38- cells compared to the normal controls ($P<0.01$). On the other hand, all of healthy controls were positive for CD38, this is in accordance with previous old study by Keyhani et al [16] who proved that high expression of CD38 indicates a high NAD+ metabolism and that CD38 is not a specific marker for blasts being expressed on a variety of cell types (for example lymphocytes and myelocytes).

In the present study, CD34 was positive in 79.8% of all AML cases, in agreement with our study, other different researchers [10, 17, 18, and 19] using the same technique detected CD34 positive expression in (57%, 61%, 65%, and 68% respectively). However, another two different studies [11, 20] were not in agreement with our results, they found a wide variation ranging between 25% and 64% for CD34 expression among AML cases (25% and 64%) and stated that this could be due to methodological variation in detection of receptor expression (like flurochrome labeling, varying gates in flow cytometric analysis, and different CD34 antibodies recognizing distinct CD34 epitopes).

No statistically significant difference was found between CD34 expression with age, or its expression with any of the hematological findings except with low PB blast count (p-value=0.05). Further studies supported our results by finding no significant correlation between the expressions of the progenitor CD34 and CD38 cell markers with age. On the contrary, the same authors also detected no correlation with any of the hematological findings including the PB blast count [10, 17]. According to the FAB subtypes, we found the highest CD34 expression among M2 subtype (41.8%) ($P=0.006$) and this was close to what was found by Mona et al. [17]. Where she found that CD34 expression was highest among M0-M1 subtypes, but it didn't reach a statistically significant value. This does not correlate with other previous studies detecting no correlation between CD34 expression and FAB subtypes [10, 19]. In agreement with a study done by Legrand et al. [19], no correlation was found between CD34 expression and the cytogenetic risk groups. On the other hand, it does not correlate with the results of Buccisano et al. [21] who confirmed a significant correlation between unfavorable karyotypes and high expression of CD34. In addition, Mona et al. [17] found that the highest percentages were detected in the poor risk group (88%) but didn't reach a statistically significant difference. So increasing our sample size may confirm or exclude such correlation. Regarding the CD38 progenitor cell marker, 85.7% of our studied AML cases were positive expressors. Other studies showed the same results among their cases or even higher frequencies for the same marker (82.5% and >95%) due to the larger sample size studied (304 cases) [10, 16, 17].

Our study demonstrated no significant association between CD38 expression with age, FAB subtypes and any of the hematological findings except with low PB blasts ($P=0.005$), this didn't go in line with others who found no significant difference among their cases with any of the hematological findings [10, 17], however in an old study reported by Keyhani et al. [16] they previously detected a significant lower expression for the CD38 marker with M3 FAB subtypes. Besides, no significant association in our cases for CD38 expression with any cytogenetic and molecular group was found. This was not in agreement with Mona et al. [17], although they found the highest percentages of CD38 expression among the poor risk group (100%) yet, no significant difference was found as the number of patients in their study might still not be sufficient to give a conclusive result. In the present study, considering the response rate, there was no statistically significant difference for both progenitor markers studied separately among our cases. This was in agreement with a study proposing that CD34 alone could not be an independent marker for prognosis and recommended apply the combination of CD34 with other markers [19]. This was discordant with other studies who reported that increased CD34and CD38 were associated with higher relapse rate, explaining this finding as the blast cells become more resistant to apoptosis with increasing CD34 proportions resulting

in bad prognosis [10]. In addition, other groups observed a decreased rate of complete remission as well as a diminished overall survival in patients with a CD34+ AML [22]. Similarly, Petrovici [10] found that patients with > 65% CD38+ blasts showed only a tendency for shorter relapse-free survival than those with < 65% CD38+ cells. Others suggested that patients with a high CD38 expression had significantly longer and higher rates of remission and a longer event-free survival [16]. Previous old studies, demonstrated that the only primitive progenitor markers CD34+ve/CD38-ve cells in AML and not the more mature one CD34+ve/CD38+ve and CD34-ve population were capable of initiating the disease [8], and since one of the major challenges in the design of new therapies to eradicate leukemia stem cells is to achieve high therapeutic specificity and as there is a complex prognostic network constituted by different markers in AML, we analyzed in our study the interaction between CD34 and CD38 markers in combination to evaluate their diagnostic and prognostic relevance with AML patients.

There was a high statistical significant differences among different groups (<0.005), (70.2%) of all our AML cases were among CD34+ve/CD38+ve group, and only, (9.5%) were CD34+ve/CD38-ve, (15.5%) were CD34-ve/CD38+ve, and (4.8%) of cases were among CD34-ve/CD38-ve group. Our results are in agreement with other two recent studies, together they confirmed that the LSC population is phenotypically diverse and can vary markedly among patient subgroups, and even between individual patients within these subgroups. However, they questioned how this might reflect the heterogeneity of the initial target cell transformed [5, 23]. Other groups as Goardon and co-workers [24] found that AML LSCs can also reside within the CD34+CD38+ or the CD34- immunophenotypic compartment as these cells (CD34+/CD38+, CD34-) probably originate from (limited) differentiation of the CD34+CD38- LSCs, a process that has been shown to occur in vivo. Another study also detected that the neoplastic component of the CD34+CD38+ and CD34- compartment represented a considerable portion of the total neoplastic blast compartment [25]. This reflects the total leukemic burden, or MRD, which, in turn, have a prognostic impact. This does not correlate with the results of another study, they have shown in their analysis that most LSCs reside in CD34+CD38- compartment, but also found a small subset of their cases among CD34+CD38+ and CD34- compartments. They stated that, this does not mean that CD34+CD38+and CD34- cells do not contain leukemia initiating ability; it simply strongly suggests that, in the presence of CD34+CD38-cells, these CD34+CD38+and CD34- leukemia initiating cells are less malignant compared to CD34+CD38- cells and recommended that In the future, it would be interesting to examine the relationships between the functional phenotypes (based on aldehyde dehydrogenase activity) and the CD34/CD38 immunophenotype [26].

Although we found a high statistical significant difference among low TLC, low HB level, low PB blast count and different FAB subtypes among CD34/CD38 groups (0.05, 0.047, 0.035, 0.002 respectively), yet, the response rate among them was insignificant (P=0.87). Our results are not consistent with that encountered by other different studies who found that the CD34+CD38- compartment is most important in the clinical setting and were more therapy-resistant and less immunogenic than other compartments [26, 27, 28], yet one of these studies stated that in AML cases with no malignant CD34+CD38- compartments, the LSCs will be located in the CD34+CD38+ and/or CD34- compartments and recommended that within these relatively large compartments, further compartmentalization will be necessary to identify the true LSC sub-compartment which likely occurs at low frequencies [26].Finally, our AML cohort showed a higher rate of mortality in the mutant FLT3 patient when compared to the wild FLT3, and similar to others there was a strong prognostic value evidenced by the higher rate of relapse, these findings are in concordance with Ghaleb et al [29] and others [30,31]

5. Conclusions

In conclusion, our preliminary results have shown that the progenitor markers CD34, CD38 expressed separately or in combination among BM samples of Egyptian AML patients, might be susceptible markers providing important clues for future studies in the early detection of resistant AML cases. Their expression had to be investigated more broadly on a wide scale with a large sample size in a long term follow up study to establish whether patients with a high expression of CD34 and CD38, should get a more intensified therapy or an early BM transplantation. So future studies will help to further validate the prognostic importance of these markers and once this is clear, it may be possible to appropriately tailor the aggressiveness of therapy needed in these patients especially after the high relapse rates among them.

Conflict of Interest

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Corresponding author
Reham A. Rashed
Hematology Unit, Clinical Pathology Department,
reham_r9@yahoo.com

References

1. Tefferi A, Thiele J, Vardiman JW: The 2008 world health organization classification system for myeloproliferative neoplasms: Order out of chaos. Can- cer 2009; 115: 3842-3847.
2. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al: Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia Net. Blood 2010; 115: 453-474.
3. Schneider V, Zhang L, Bullinger L, Rojewski M, Hofmann S, Wiesneth M, et al: Leukemic stem cells of acute myeloid leukemia patients carrying NPM1 mutation are candidates for targeted immunotherapy. Leukemia 2014; 8: 1759-1762.
4. Bacher U, Zander AR, Haferlach T, Schnittger S, Fehse B, Kröger N: Minimal residual disease diagnostics in myeloid malignancies in the post-transplant period. Bone Marrow Transplant 2008; 42: 145-157.
5. Taussig DC, Vargaftig J, Miraki-Moud F, Griessinger E, Sharrock K, Luke T, et al: Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. Blood 2010; 115: 1976–1984.
6. Pearce DJ, Taussig D, Simpson C, Allen K, Rohatiner AZ, Lister TA, et al: Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem cells 2005; 23: 752–760.
7. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al: A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367: 645–648.
8. Bonnet D, Dick JE: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature medicine 1997; 3:730–737.
9. Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, et al: Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. J Clin Invest 2011; 121: 384– 395.
10. Petrovici K, Graf M, Reif S, Hecht K, Schmetzer: Expression profile of the progenitor cell markers CD34, CD38 and CD90 in acute myeloid leukemia and their prognostic significance. Journal of Cancer Molecules 2010; 5: 79-86.
11. Oyan AM, Bo TH, Jonassen I, Ulvestad E, Gjertsen BT, Kalland KH, Bruserud O: CD34 expression in native human acute myelogenous leukemia blasts: Differences in CD34 membrane molecule expression are associated with different gene expression profiles. Cytometry Part B (Clinical Cytometry) 2005; 64: 18-27.
12. Van Stijn A, van der Pol M, Kok A, Bontje P, Roemen G, Beelen R, Ossenkoppele GJ, et al: Difference between CD34+ and CD34- blast compartments in apoptosis resistance in acute myeloid leukemia. Hematologica 2003; 88: 497-508.
13. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009; 114: 937-951.
14. Zhi L, Wang M, Rao Q, Yu F, Mi Y, Wang J: Enrichment of N-Cadherin and Tie2-bearing CD34+/CD38-/CD123+ leukemic stem cells by chemotherapy-resistance. Cancer Lett 2010; 296: 65-73.
15. Wen D, Yanjie H, Cong L, Juan L, Wei L, Yanli H, et al: Cluster of differentiation 96 as a leukemia stem cell-specific marker and a factor for prognosis evaluation in leukemia. Molecular and Clinical Oncology 2015; 3: 833-838.
16. Keyhani A, Yang H, Jendibora D, Pagliaro L, Cortez J, Pierce S: Increased CD38 expression is associated with favorable prognosis in adult acute leukemia. Leuk Res 2000; 24: 153-159.
17. Mona AI, Sherin MH: Prognostic Significance of Progenitor Cell Markers in Acute Myeloid Leukemia, Life Science Journal 2011; 8: 680-686.
18. Chang H, Salma F, Yi Q, Patterson B, Brien B, Minden M: Prognostic relevance of immunophenotyping in 379 patients with acute myeloid leukemia. Leuk Res 2004; 28: 43-48.
19. Legrand O, Perrot JY, Baudard M, Cordier A, Lautier R, Simonin G, et al: The immunophenotype of 117 adults with acute myeloid leukemia: proposal of a prognostic score. Blood 2000; 96: 870-877.

20. Basso G, Lanza F, Orfao A, Moretti S, Castaidi G: Clinical and biological significance of CD34 expression in acute leukemia. J Biol Regul Homeost Agents 2001; 15: 68-78.
21. Buccisano F, Rossi FM, Venditti A, Del Poeta G, Cox MC, Abbruzzese E, et al: CD90/Thy-1 is preferentially expressed on blast cells of high risk acute myeloid leukemias. British Journal of Hematology 2004; 125: 203-212.
22. Heuser M, Wingen L, Steinemann D, Cario G, von Neuhoff N, Tauscher M, et al: Gene expression profiles and their association with drug resistance in adult acute myeloid leukemia. Haematologica 2005; 90: 1484-1492.
23. Sarah JH, Brian JP: Recent advances in acute myeloid leukemia stem cell biology. Hematologica 2012; 97: 966-974.
24. Goardon N, Marchi E, Atzberger A, Quek, L, Schuh A, Soneji S, et al: Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer cell 2011; 19: 138-152.
25. Terwijn M, Van Putten WLJ, Kelder A, Van der Velden VHJ, Brooimans RA, Pabst T, et al: High Prognostic Impact of Flow Cytometric Minimal Residual Disease Detection in Acute Myeloid Leukemia: Data From the HOVON/SAKK AML 42A Study. Journal of clinical oncology 2013; 31: 3889–3897.
26. Terwijn M, Zeijlemaker W, `le Kelder A, Rutten AP, Snel AN, Scholten WJ, et al: Leukemic Stem Cell Frequency: A Strong Biomarker for Clinical Outcome in Acute Myeloid Leukemia. PLOS ON 2014; 9.
27. Costello RT, Mallet F, Gaugler B, Sainty D, Arnoulet C, Gastaut JA, et al: Human acute myeloid leukemia CD34+/CD38- progenitor cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis, reduced immunogenicity, and impaired dendritic cell transformation capacities. Cancer Res 2000; 60: 4403– 4411.
28. Bachas C, Schuurhuis GJ, Assaraf YG, Kwidama ZJ, Kelder A, Wouters F, et al: The role of minor subpopulations within the leukemic blast compartment of AML patients at initial diagnosis in the development of relapse. Leukemia 2012; 26: 1313–1320.
29. G. Elyamany, M. Awad, K. Fadalla, M. Albalawi, M. Al Shahrani, A. Abdulaaly. Frequency and Prognostic Relevance of FLT3 Mutations in Saudi Acute Myeloid Leukemia Patients. Advances in Hematology 2014, 2, ID: 41360, 1-7
30. Y. Y. Xu, L. Gao, Y. Ding et al., "Detection and clinical significance ofFLT3-ITD gene mutation in patients with acute myeloid leukemia," Zhongguo Shi Yan Xue Ye Xue Za Zhi, vol. 20, no. 6, pp. 1312–1315, 2012.
31. C. Thiede, C. Steudel, B. Mohr et al., "Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis," Blood, vol. 99, no. 12, pp. 4326– 4335, 2002.

3/26/2016