Prognostic Significance of Progenitor Cell Markers in Acute Myeloid Leukemia

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Abstract: Background: Until now the prognostic significance of flow cytometric immunophenotyping (FCI) in acute myeloid leukemia (AML) has been controversial. The decision whether patients with AML should receive a more intensified therapy has been made according to defined risk categories based mainly on genetic criteria. Unfortunately no specific chromosomal abnormalities are found in about half of the patients. So additional prognostic factors are needed. Aim of work: The aim of the current work was to investigate prognostic value of progenitor cell markers CD34, CD38 and CD90 expression on AML blast cells at initial diagnosis, and to correlate this expression with known prognostic parameters as well as with the clinical outcome.   Patients &Methods: This work was conducted on 80 patients with de novo AML meeting World Health Organization criteria for AML, FAB subtype M0-M5 were included. The levels of progenitor markers were determined by FCI, corresponding cytogenetic results were obtained, appropriate follow-up information were analyzed. Results: Sixty one percent, 82.5% and 35% out of 80 patients were positive for CD34, CD38 and CD90 respectively. No differences in expression were found in different FAB subtypes and cytogenetic risk groups. Cut off values were calculated with values ≥38 for CD34, ≥55 for CD38 and ≥52 for CD90. A significant high resistance to induction therapy and poor outcome were observed in patients with increased progenitor cell expressions. Conclusion: Progenitor cell markers are sensitive indicators as regard response to therapy and clinical outcome in patients with de novo AML. Therefore, their determination should be taken into consideration when designing therapeutic regimens.

1. Introduction

Acute myeloid leukemia is an aggressive malignancy characterized by accumulation of immature myeloid progenitor cells in the bone marrow [1]. The majority of the adult patients (70-80%) with de novo AML will achieve an initial complete remission after chemotherapy. However, long term free survival remains as low as 30-50% [2]. Moreover, there are poor prognostic groups who are less probable to achieve complete remission with induction treatment and for whom the overall survival is less than 1 year [3].

The growth and differentiation of the progenitor cells are regulated by specific cytokines and growth factors and their corresponding receptors. By analyzing those receptors it is possible to determine the grade of differentiation and the lineage of the progenitor cells [4].

CD34 is expressed on the surface of immature hematopoietic normal progenitor cells that compromise 1-2% of the cells [5]. It is not lineage restricted and thus not useful for distinguishing AML from ALL [6]. In addition, CD34 is involved in cellular adhesion and mediates resistance to apoptosis [1]. CD34 AML blast cells are even more resistant to programmed cell death with increased percentages of CD34 cells [7].

CD38 is mostly expressed on the surface of immature cells and different lineages of hematopoietic activated cells like lymphocytes and myelocytes [8]. 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 [9].

CD90, also known as THY-1, is a 25-35 KDa, glycosylphosphatidyl inositol (GPI) linked surface protein expressed on primitive hemopoietic stem cells in normal BM, cord blood and fetal liver cells. The function of CD90 is not yet clear but possibly it is responsible for hemopoietic cell adhesion and recognition [10].

The aim of this study was to investigate prognostic role of progenitor cell markers CD34, CD38 and CD90 expression on AML blast cells at initial diagnosis, and to correlate this expression with known prognostic parameters as well as with the clinical outcome.

2. Subjects and methods

This study included 80 patients with de novo AML presented to Hematology/Oncology Clinic, Ain Shams University Hospitals in the period from January 2009 to March 2011. Their ages ranged from 21-73 years with a mean of 35.28±14.01 years, with a male to female ratio 2:1.

All patients were subjected to thorough history and clinical examination, complete blood picture (using LH 750 Coulter, Beckman) and BM aspiration with examination of Leishmans' stained blood smears, immunophenotyping for estimation of CD34, CD38 and CD90 (using Epics XL flow cytometry, Coulter, Electronics, Hialiah, Fl, USA).

Diagnosis and classification of leukemic cases were based on morphology and cytochemistry according to the French American British (FAB) classification and meeting World Health Organization criteria for AML , immunophenotyping results and corresponding cytogenetic results were obtained, appropriate follow-up information were included.

Flow cytometric immunophenotyping of blast cells was performed using whole blood lysis method. A panel of mouse monoclonal antibodies directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE0 or tandem Cy 5-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) as well as PE labeled anti CD34, FITC labeled anti CD38, (Beckmann Coulter, Krefeld, Germany), and PC5 labeled anti CD90 (BD Biosciences, Mountain View, CA, USA). An appropriate isotype control IgG1 was used in all cases to assess background fluorescence intensity.

The blast population was gated according to their FS and SS. 5000-10000 cells in the gate were analyzed. If the percentage of positive events was >20%, the leukemic sample was considered as positive for that surface marker [11] as well as progenitor cell markers [12], except for CD34 and intracellular MPO where expression more than 10% was considered positive [13].

Conventional cytogenetic analysis: 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 hypotonic wash and fixative then 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 an image system cytovision/genus application soft ware versus 2.7 (UK).

Statistical analysis:

Data were collected, verified, revised and then edited on PC. Then data were analyzed using IBM SPSS statistics (V.19.0, IBM Corp., USA). Association of categorical data parameters was performed using Chi square test, Fisher exact was performed for value less than 5. Mann-Whitney U test was used for unpaired data The receiving operating characteristic (ROC) was performed to calculate the cutoff values. Kaplan Meier technique was used to estimate the overall survival. A P value <0.05 was considered significant and <0.01 highly significant.

3. Results:

Results of the present study are presented in Tables 1-3 and Figures 1-2.

The study was conducted on 80 patients with de novo AML classified according to the FAB and immunophenotypic criteria. The blast cells were identified according to their forward and side scatter or CD45 expression and side scatter (SS), electronically gated and analyzed by flow cytometry (Figure 1).

According to the age, the patients were divided into 2 groups, patients <60 years and patients >60 years. The frequency of progenitor cell markers were higher in patients who were older than 60 years when compared to patients <60 years. However, these differences were statistically non significant (p >0.05) (Table 1).

Hepatomegaly, splenomegaly, lymphadenopathy, peripheral blood and bone marrow blasts were not significantly associated with progenitor cell markers expression (data not shown).

As regards CD34, fifty two out of 80 patients (61%) were positive and was mostly expressed in immature AML M0 and M1(87.5% and 77% respectively). It was less in other FAB subtypes. However, these differences were statistically non significant with a P value >0.05.

CD38 was positive in 66 out of 80 (82.5%) patients and highly expressed in all FAB subtypes, however this expression was not significant regarding FAB subtypes (P value <0.05).

Twenty eight patients (35%) out of 80 patients with AML were positive for CD90 expression. CD90 showed highest percentage with M3 (55%) but regarding the FAB this difference was statistically non significant.

Successful mitosis was encountered in 74/80 (92.5%) of cases. 29/74 (39%) were classified as favorable group {t(8;21); t(15,17) or inv 16}, 19/74 (26%) were classified as intermediate with normal karyotype or trisomy 8 and 26/74 (35%) were classified as poor cytogenetic risk group with t(9,11) or del11q23.

Studying the association between cytogenetic and progenitor markers revealed that all progenitor markers were higher with adverse risk groups than favorable one, however this difference wasn't

statistically significant (Table 1).

Using the receiver operating characteristic (ROC) study, cut off values of the progenitor cell markers for the most significant separation and differentiation between cases with relapse/death or remission were calculated. Most significant differences between the cases with complete remission and inferior outcome were found in patients with a cut off value more or less than 38% for CD34, 55% for CD38 and 52% for CD90.

There was a highly significant relation between progenitor markers expression and response to chemotherapy (p<0.01), where patients with increased expression were mostly non responders to

chemotherapy, while most patients with expression less than cut off values responded to chemotherapy (Table 2).

Patients were followed up for 15 months to detect patient outcome. By using Kaplan Meier curves, 76% of patients with CD34 expression <38 showed free survival, while only 25% of the patients with expression >38were still in remission. In addition, 67% of patients with CD38 expression ≥55% and 69% of the patients with CD90 ≥52% relapsed after 12 months follow up (Table 3, Figure 2).

Table 1: Association between CD34, CD38 and CD90 and clinical features in AML patients.

	No of patients	CD34% Positive	CD38% Positive	CD90% Positive	Sig
Age <60years >60years	32 48	25 27	25 41	12 16	NS
Sex Male Female	41 39	24 28	36 30	15 13	NS
Cytogenetic Favorable Intermediate Poor	29 19 26	13 16 23	19 21 26	6 9 13	NS
FAB M0 M1 M2 M3 M4 M5	8 13 29 18 8 4	7 10 19 10 4 2	7 11 25 15 5 3	2 3 7 10 4 2	NS

Table 2:Association between progenitor markers expression and response to chemotherapy in all studied patients.

	CD34 <38		CD34 ≥38		CD38 <55		CD38 ≥55		CD90 <52		CD90 ≥52	
	No	%	No	%	No	%	No	%	No	%	No	%
Responders	22	76	13	25	23	66	6	9	24	63	11	26
Non responders	7	24	38	75	12	34	25	81	14	37	31	74
Sig.	HS				HS				HS			

Table 3: Association between progenitor markers and patients outcome

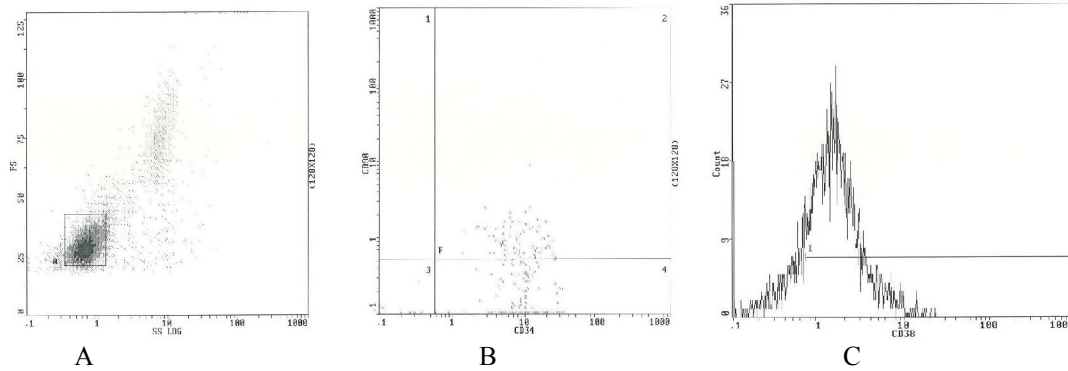| Clinical outcome	CD34 <38		CD34 ≥38		CD38 <55		CD38 ≥55		CD90 <52		CD90 ≥52	
	No	%	No	%	No	%	No	%	No	%	No	%
CR	22	76	13	25	19	54	12	39	24	63	11	26
Relapse	6	21	31	61	14	40	18	58	10	26	29	69
Death	1	3	7	14	2	6	1	3	4	11	2	5

Figure1: Flow cytometric analysis of an AML case. A: shows gated blasts, B: shows CD34 and CD90 expression and C: shows CD38 expression.
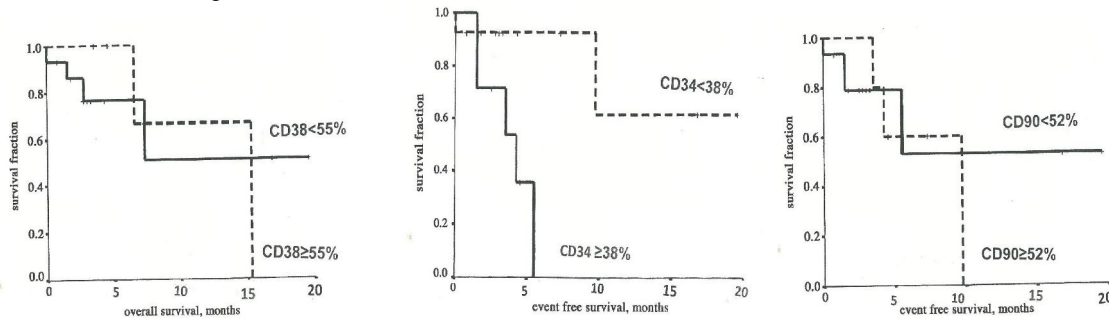

Figure 2: Kaplan –Meier curves for prognostic significance of progenitor cell markers in AML patients.

4. Discussion

The major prognostic parameters with regard to relapse of AML are the response to induction chemotherapy and the genetic abnormalities of the malignant cells[14,15]. It is still not known whether AML cell expressions of progenitor cell markers CD34, CD38 and CD90 represent an additional independent prognostic factor [16].

The present study was carried out on 80 patients with de novo AML, aiming to assess the prognostic value of progenitor cell markers in AML and to establish a relationship between them with the response to chemotherapy and clinical outcome. These markers were established by flow cytometry .

Flow cytometric analysis improves both accuracy and reproducibility of the FAB classification and is considered to be practically useful for the detection of MRD by monitoring AML patients in remission [12].

In this study CD34 was expressed in 61% of the patients with AML, these data were consistent with other studies using the same technique who detected 65% expression of CD34 [17]. This was also in agreement with **Petrovici et al., 2010** [21] and Legrand **et al., 2000** [18] who stated that the expression of CD34 was 57% and 68% respectively.

However, wide variation ranging between 25% and 64% [1] was encountered by **Basso et al., 2001** [19] , that 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).

CD34 expression was highest in M0-M1 FAB subtypes, however this was not statistically significant. This was consistent with other studies [12] who detected no correlation between CD34 expression and FAB subtypes.

Also no significant difference was detected between this progenitor cell marker and age as well as other clinical and laboratory parameters. This was in agreement with other investigators [14,20] who detected no correlation between CD34 expression and clinical data of the patients.

In this study, CD38 expression was expressed in 82.5% of studied AML patients. This expression was lower than that detected by **Keyhani et al., 2000** [21] who detected CD38 expression in >95% of AML cases with 63%-83% positive cells. The different percentages could be due to a lower case number studies as compared to 304 cases studied by them. Furthermore, CD38 is not a specific marker for blasts

being expressed on a variety of cell types (for example lymphocytes and myelocytes) [21].

Our study demonstrated no significant association between CD38 expression with different FAB subtypes or age. This was in agreement with other studies [12] that detected no significant association. On the other hand, **Keyhani *et al.,* 2000** [21] found a significant lower expression of CD38 with M3 FAB subtypes.

Regarding CD90 expression, 28/80(35 %) expressed CD90. This was consistent **Filler *et al.,* 2008** [22] who found coexpression of CD34 and CD90 in 42 out of 120 cases (35%). However, **Buccisano *et al.,* 2004** [23] found an overall frequency of CD90/CD34 positive cases were 17% and that CD90 was always expressed in the CD34 positive cell fraction. On the contrary, other studies [24] found higher percentage than this study which may be explained by their lower reference value e.g. 5% of all positive cells chosen as cut off between CD90+ and CD90- cases.

Again **Petrovici *et al.,* 2010** [12] found the expression of CD90 in 66% of investigated AML case, when measuring CD90+on CD34 + double positive cells (on de novo and secondary AML) using FITC conjugated anti CD90 monoclonal antibody with PE labeled anti CD34 monoclonal antibody.

In our study, the highest CD90 expression was found in AML-M3 (55%), yet no significant difference could be detected between FAB subtypes. This was in agreement with other studies [12] that found the expression of CD90 highest with M3 subtype but still statistically non significant.

There was no statistical significant difference was found between progenitor cell markers and cytogenetic results, although the highest percentages were detected in the poor risk group (88%, 100%, 50% in CD34, CD38 and CD90 respectively) (Table 1). However, the number of patients in this study might still not be sufficient to give a conclusive result.

This was similar to other results [18] that didn't find a correlation between CD34 expression and cytogenetic risk groups. On the contrary other results [23] confirmed a significant correlation between unfavorable karyotypes and high expression of CD34 and CD90.

AML cut off values were identified in order to allow the most significant separation and differentiation between AML cases with remission or relapse/death. In this study patients with more than 38% for CD34,   55% for CD38 and   52 % for CD90 were associated with poor response to chemotherapy while most patients with less expression responded to chemotherapy with a highly significant difference between both groups.

After a 12 month follow up, regarding patients with CD34 more than cutoff value, 61% relapsed, 14% died and only 25% developed remission (Table 3). In addition, patients with CD38 and CD90 more than cutoff values showed 67% and 69% relapse, 2% and 5% died and 31% and 26% developed remission respectively.

This was in agreement with other studies[12] that stated that increased CD34 and CD38 were associated with increased relapse rate. A possible explanation could be that blast cells get more resistant to apoptosis with increasing CD34 proportions resulting in bad prognosis [7]. In contrast, another study proposed that CD34 alone could not be an independent marker for prognosis. It was recommended using a combination of CD34 with other markers [18].

This was also in agreement with **Buccisano *et al.,* 2004** [23] who found that AML patients characterized with poor prognosis (such as elderly AML, de novo AML with unfavorable cytogenetic or drug resistance) were significantly associated with high CD90 expression. On the contrary **Petrovici *et al.,* 2010**[12] found that patients with increased CD90 expression on the blast cells were associated increased remission.

Regarding cytogenetic analysis, our findings support previous reports [25,26] ascribing poor prognosis to AML patients positive for t(9,11) or del11q23, favorable prognosis to AML patients positive for t(8,21), t(15,17) or inv 16.

From this study we can conclude that the expression of progenitor markers CD34, CD38 and CD90 could be used as a predictor of poor therapeutic response and relapse in   de novo AML. In this regard, for the consideration of whether patients with high expression of CD34, CD38 and CD90 should get more intensive consolidation therapy or an early bone marrow transplantation due to their poor prognosis, Further studies on wide scale de novo AML patients should be implemented. In addition, in cases with post remission targeted therapies as immunotherapies or in cases with intermediate risk karyotype the prognostic impact of progenitor cell markers could contribute to refine adapt these protocols for individual patients.

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