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RESEARCH ARTICLE

Significance of CD200 expression in patients with acute myeloid leukemia and its correlation with other prognostic markers

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ABSTRACT

Background and Objectives: Acute myeloid leukemia (AML) known as cancer of blood and bone marrow is regarded as the commonest acute leukemia in adult patients. characterized by uncontrolled proliferation of hematopoietic progenitor cells with arrest of maturation and disruption of normal hematopoiesis. CD200 is a protein belonging to the immunoglobulin superfamily, it has an immunosuppressant effect by interacting with its receptor (CD200R). **Aim:** The main aim of this study is to investigate the expression of CD200 on leukemic myeloid cells. Various molecular prognostic markers and other clinical and laboratory findings were studied in relation to CD200 expression. **Patients and Methods**: The present study was conducted on newly diagnosed AML patients attending the adult Hematology/Oncology Clinic of the National Cancer Institute. The expression of CD200 was determined by flow cytometry using anti-CD200 monoclonal antibodies. CD200 expression considered positive if ≥20% and negative if <20%. **Results**: CD200 positive expression was found in 62/104 (59.5%) patients, CD200 was more expressed in CD34 positive cases (P= 0.012) and cases with gum hyperplasia (P= 0.046). FLT3/ITD mutation and NPM mutation were less detected in AML patients with positive CD200 (p=0.045 and p= 0.036 respectively). We found a statistically significant relation between inv16 and CD200 positive expression (p=0.005). **Conclusion**: CD200 could be used as a biological biomarker in AML pathophysiology, and could be incorporated in the initial diagnostic workup in patients treated within clinical trials for the discovery of new therapy which target malignant leukemic cells without harming other cells in AML.

Keywords: Acute myeloid leukemia AML; CD200; Imunophenotyping.

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INTRODUCTION

Acute myeloid leukemia (AML) is the malignancy of the blood and bone marrow and is considered the most common acute leukemia in adult patients (Kouchkovsky et al., 2016). Its incidence is more in patients aged 60 years (Roman et al., 2016). And more in males (Juliusson et al., 2017). The last World Health Organization (WHO) classification 2016 identifies distinct categories of AML (Arber., 2016). With advanced age, the relative incidence of AML with recurrent genetic abnormalities decreases (Bullinger et al., 2017), while the relative incidence of other AML categories increases with age (Ostgard et al., 2015).

Besides clinical and laboratory data initially at diagnosis, as age, performance status, tumor burden, prior hematological disorder or exposure to chemotherapy or radiotherapy and extramedullary disease (Greenwood et al., 2006**.** Smith et al., 2011), many cytogenetic as well as molecular abnormalities are currently used for risk stratification (Grimwade et al., 1998- Schlenk et al., 2008). The genetic features of AML and understanding the leukemia biology are important to move towards a patient tailored therapy and follow-up (Dohner et al., 2010**,** Estey, 2014)

Most patients with AML after induction chemotherapy achieve remission, but relapse and resistance to chemotherapy occur.

Leukemia and the relapsed leukemia are hypothesized to arise from LSCs, eleminating LSCs is the critical target for curing AML (Thomas et al., 2017). Stem cell transplantation provides a proof that AML LSCs can be eliminated by a functional immune system (Stelljes et al., 2014). Immunophenotyping done for AML patients provides information for the diagnosis, classification, and monitoring of AML. Also, it helps to identify, quantify and assess the lineage of leukemia blast cells and disease classification according to the maturation stage (Finak et al., 2016).

CD200 (Cluster of differentiation 200) is a type I immunoglobulin superfamily membrane glycoprotein. CD200 is normally expressed on neurons, endothelium, thymocytes, and populations of B- and T-lymphocytes (Barclay et al., 2002) and is involved in immune responses regulation through interaction with its receptor (CD200R) that is expressed on immune cells (Gorczynski, 2012). A high CD200 expression has been associated with poor clinical outcomes in patients with AML (Tiribelli et al., 2017). The function of CD200 in normal and malignant hematopoiesis is unclear, however it mostly has an immunomodulatory role. Expression of CD200R is limited to immune cells, predominantly myeloid cells and subsets of natural killer, B, and T cells (Manich et al., 2019).

The result of CD200–CD200R interaction has immunosuppressive effects that influence responses to pathogens, autoimmunity, transplant tolerance, and cancer surveillance (Gorczynski, 2012). Also in the hematopoietic system, CD200–CD200R signaling may minimize the autoimmunity against HSCs in healthy individuals; however, LSCs may exploit this mechanism.

Several studies were conducted to evaluate the targeting of the CD200-CD200R immune checkpoint by an antiCD200 monoclonal antibody in patients with leukemia (Mahadevan et al., 2019). Some of them showed immunosuppression in AML is associated with changes in the adaptive immune response (Liepert et al., 2010), and others are conducted to evaluate the concept of targeting CD200 on LSCs in target to reduce relapse rates and improve the outcomes in AML (Ho et al., 2020). So, we aimed in this study to investigate the expression pattern of CD200 on myeloid blast and blast equivalent cells and to determine whether CD200 could emerge as a prognostic marker. Relation of CD200 expression with various clinical and laboratory findings was attempted. For the possibility of using CD200 as an immune target in AML therapy.

MATERIALS AND METHODS

Study population

Our study included 104 newly diagnosed (de novo) Acute Myeloid Leukemia cases. Their age ranged from 18 years to 60 years. They were 41 (55.4%) females and 33 (44.6%) males. They all presented to the Adult Hematology/oncology outpatient clinics, National Cancer Institute, Cairo University, during the time period from March 2017 till February 2018. This study was approved by the ethical committee, and review board of National Cancer Institute, Cairo University in accordance with Helsinki guidelines for the protection of human subjects. Written informed consent was obtained from all patients.

All cases were subjected to the following: Full history taking, clinical examination and Laboratory investigations including: Complete blood count (CBC), BM aspiration and examination, Immunophenotyping using Acute Leukemia panel which includes (Pan leucocytic marker: CD45, Myeloid markers: CD13, CD33, cyt MPO, cyt CD13 and CD117, Monocytic markers: CD14, CD64 and CD11c, Common progenitor marker: CD34 and HLA-DR, Lymphoid markers: CD19, CD22, CD10, CD2, mCD3, CD5, CD7, CD4 and CD8), Conventional karyotyping and Molecular studies. All patients of this study were treated according to The National Cancer Institute, Cairo University's ongoing induction and consolidation regimens for treatment of adult AML cases.

Received *induction chemotherapy* with a combination of 7 days of cytosine arabinoside (100 mg/m2) and 3 days of Adriamycin (45 mg/m2). Patients who achieved complete remission (CR) and had favorable cytogenetics [inv16 and t (8; 21)]: Received *consolidation chemotherapy* with high dose Ara-C (3gm/m2 IV infusion over 3 hours/12 hours for 3 days) for a

total of 3-4 cycles. Patients with high-risk cytogenetics (monosomy 7 or 5, deletion of 5q and abnormalities of 3q and those with a complex karyotype) or intermediate-risk cytogenetics (those with normal cytogenetics and other changes not associated with high risk or favorable groups): were transferred for *allogenic bone marrow transplantation* if they had matched sibling donor after achieving CR. Patients who did not have matched donors: Received *consolidation chemotherapy* as the in favorable group. Patients who relapsed after conventional chemotherapy or failed to achieve CR despite optimal induction treatment: Received second *induction* and then were transferred for *allogenic bone marrow transplantation* if they had matched sibling donor. Patients who relapsed after bone marrow transplantation: Received *palliative chemotherapy* (as HAM: High dose ARA-C, Mitoxantrone).

Follow up of patients' data during the course of treatment had been done by assessing the response to treatment clinically and by bone marrow examination at day 14 and 28 and the impact on survival. Complete remission (CR) was defined in accordance with standard criteria by Dohner et al. which required an absolute neutrophil count of 1.5 x 10^9/L, a platelet count of 100 x 10^9/L or more, no blasts in peripheral blood (PB), BM cellularity more than 20%, no Auer rods, less than 5% BM blasts and no extramedullary leukemia (Dohner et al., 2010). Disease-free survival (DFS) for our patients was measured from the date of CR to the date of relapse or death from any cause. The overall survival (OS) for our patients was measured from the date of diagnosis until the date of death, censoring for patients alive at the last follow-up.

Detection of CD200 expression on myeloblasts by flow cytometric analysis (Figures 1, 2)

One ml bone marrow sample was collected from patients through bone marrow aspiration under complete aseptic conditions. Bone marrow was delivered to a vacutainer tube containing EDTA for Flow- cytometric analysis of leukemic myeloid blast cells using CD45 and CD200. The expression of CD200 on the surface of blast cells was determined by Flow cytometer 10 colors BD FACS Canto. and analyzed by FacsDIVA software.

The analysis was performed using direct staining method and the following monoclonal antibodies were used: Fluorescein- conjugated (PE- phycoerythrin), mouse / IgG1, Kappa subclass, monoclonal anti-human CD200 (membrane glycoprotein, OX2) purchased from BD Pharmingen™ (Cat. No: 552475), (clone: MRC OX-104). And Fluorescein- conjugated PE-Cy5 (phycoerythrin- cyanine 5) mouse / IgG1, Kappa subclass, monoclonal anti CD45 purchased from BD Pharmingen™ (Cat. No: 555484), (clone: HI30).

A logarithmic scale was implemented for forward scatter signal, side scatter signal and each fluorescent channel. Data analysis was performed as follows: about 20,000 events were analyzed, primary gate was constructed on CD45 dim population and measuring the expression of CD200 as a percent within the primary gate.

Interpretation of the results: After 20,000 events were counted, the percent of CD200 expression was calculated against CD45 dim population. A cut-off value of 20% was set to categorize samples as positive (Damiani et al., 2015)._Immunophenotyping of bone marrow myeloblasts by gating on CD45 dim population (red color) revealed positive expression of CD200. Lymphocyte population which is CD45 bright (green color) used as an internal positive control. Both populations are overlapping (same pattern of expression).

Immunophenotyping of bone marrow myeloblasts by gating on CD45 dim population (red) revealed positive expression of CD200. Lymphocyte population which is CD45 bright (green) used as the internal positive control. Myeloblasts showed dimer expression for CD200 than lymphocytes**.**

Statistical analysis

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as median and range according to the performed normality tests. Qualitative data were expressed as frequency and percentage. Chi-

square test or Fisher's exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. All tests were two-tailed. A p-value < 0.05 was considered significant.

RESULTS

Patients' characteristics

This study included 104 cases of newly diagnosed AML presented to the medical oncology department of National Cancer Institute (NCI), Cairo University, 48 males (46.2%) and 56 females (53.8%). The age ranged from 19 to 70 years with a median of 39 years. Hepatomegaly was encountered in 11 patients (10.6 %), spleen was enlarged in 11 patients (10.6 %), lymphadenopathy was found in 18 patients (17.3 %) and gum hyperplasia was found in 10 patients (9.6%). Hematological findings at the time of diagnosis; CBC showed: TLC ranged from 1.06-351.00 ($x10^3$/ul), Hb ranged from 3.7 to 13.6 (g/dl), Platelets count ranged from 1 to 496 ($x10^3$/ul) and blasts ranged from 7 to 99%. Bone marrow aspiration showed: 81.7% of the patients with hypercellular marrow, Bone marrow blasts ranged from 20 to 100% with median value 75%.

Immunophenotyping studying showed: 42.3, 38.5, 8.7, 9.6 and 1% of cases presented with myeloid, myelomonocytic, monocytic, myeloid with aberrant CD7 and myeloid with aberrant CD19 phenotype respectively. Cytogenetic analysis was done to 56 patients, 20 showed recurrent cytogenetic abnormality (t(8;21), inv16, t(9;22) in 5,10, 5 patients respectively), another 11 patients showed other cytogenetic abnormalities (+8, -10, +14,-17, -18, +22) while 5 patients with complex karyotype (showing 3 or more cytogenetic aberrations). By conventional PCR FLT3/ITD heterozygous mutation was found in 24/98 patients. NPM mutation was found in 7/35 patients. By RT-PCR: t(8;21), inv16 and t(9;22) was positive in 5/98, 10/98 and 5/98 of AML patients respectively. The patients were classified according to genetic risk (combined cytogenetic and molecular analysis was assigned according to Dohner et al., 2010 into three risk groups 19: 12 patients with favorable prognosis. 66 patients were included in the intermediate risk group. 23 patients showed poor prognosis, 3 cases could not be included in this classification due to lack of their data (Table 1).

CD200 expression in AML

Analysis of CD200 expression was done in 104 patients. CD200 expression ranged from 0.1 - 99.90%, with a median 49.1500% and MFI ranged from 0-16.2 with a median 1.255. CD200 expression was considered positive if ≥20% and negative if <20%. 62/104 (59.5%) patients showed positive expression for CD200 (≥20%) and 42/104 (40.4%) patients were negative (<20%) (Table 3).

Relation between CD200 expression with different parameters

Comparing the clinical and the laboratory findings in patients with CD200 positive versus patients with CD200 negative: There was a significant association between CD200 expression and patients'age as the median age of the positive CD200 patients is 37 years and the median of the negative CD200 patients is 50 years old (p=0.002), also a statistically significant relation was found between patients with gum hyperplasia and CD200 expression (P=0.046) (Table 4). Regarding immune-phenotypic analysis There was a statistically significant relation between CD200 expression and CD34 and cytoplasmic CD13 positive expression (P= 0.012, 0.029 respectively). Molecular analysis revealed that FLT3/ITD mutation and NPM mutation were more detected in AML patients with negative CD200 patients than in AML patients with positive CD200 expression (P=0.045 and 0.036 respectively), while there was a strong statistically significant relation between inv16 and CD200 positive expression (p=0.005) (Table 5).

Comparing the treatment outcome between the patients in relation to positive and negative CD200 expression: 40/91 patients achieved complete

remission (CR) at 28th day of chemotherapy, 28 of them were positive to CD200 (50% of CD200 positive patients) versus 12 were negative to CD200 (34.3% of CD200 negative patients) (p=0.29). At last, follow up: 62/104 patients died, 41 patients were alive, of patients with CD200 positive expression 31/62 were alive and 31/62 died versus 31/42 died and 11/42 were alive of patients with CD200 negative expression (p=0.024).

Survival analysis

Kaplan – Meier plots comparing AML patients with initial positive and negative CD200 expression. Results revealed that there was a statistically significant relation between CD200 expression and overall survival (P = 0.007) (Table 6, Figure 3). Regarding the impact of CD200 expression on disease-free survival, results revealed that there is no statistically significant relation between CD200 positive expression and long disease-free survival (P = 0.912) (Table 7, Figure 4). Disease-free survival is calculated from the date of CR till the date of relapse, death or the last follow-up, those who did not achieve CR, time of disease-free survival will be equal to zero.

DISCUSSION

Previous studies showed that AML patients with high CD200 expression exhibited reduced Natural Killer and T cell immune responses which indicate that CD200 is an immunotherapeutic target in AML. Also, it was suggested that CD200 can be responsible for immune evasion and relapse after treatment in AML **(**Coles et al., 2011- Coles et al., 2015). Other studies suggested that CD200 can be a marker for LSCs that are responsible for relapse in AML and its targeting can benefit patients with refractory or relapsed AML disease (Ho al., 2018).

Previous studies suggested that blocking of CD200 by using anti-CD200 antibody treatment leads to inhibition and suppression of the interaction between CD200 and CD200R, that activates patient NK cells restoring adaptive and innate immune response for destruction of tumor cells (Memarian et al., 2013). A recent study shown that anti-CD200-blocking antibody (TTI-CD200) treatment enhanced the function of autologous immune cells in vivo and significantly improved efficacy of adoptive immune effector (Cytokine Induced Killer) CIK cells towards residual AML cells in vivo (Rastogi et al., 2020).

In respect to analysis of CD200 expression in 104 newly diagnosed AML patients in our study: CD200 positive expression (≥20%) was frequently detected in our patients (59.5%) patients. In comparison to 76.5% reported by Atfy et al., 2015 and 56%, 48%, 43%, 53.3% detected in studies by Damiani et al., 2015- Tiribelli et al., 2017- Tonks et al., 2007 and Muhsin et al., 2017 respectively in AML. Also, in patients with precursor leukaemia lymphoma it was found CD200 positive expressed in (66%, 95 and 80.3%) reported by Aref et al., 2018- Alapat et al. 2012 and Awad et al, 2016. We can suggest CD200 be used as a minimal residual disease marker as well as for targeted antibody therapy, anti-CD200 antibody direct to $CD200^{+}$ AML cells, this antibody-based therapy can stimulate cell phagocytosis to increase the possibility of AML curing and to limit AML relapse.

Referring to extramedullary manifestations in our study, we found a significant relation between positive expression of CD200 and gum hyperplasia, which is a common presentation in AML with monocytic differentiation. Thereby CD200 positive expression was found to be more in AML with myelomonocytic and monocytic differentiation compared to myeloid phenotype leukemia. Moreover, CD200 was significantly expressed more with inv (16) positive cases (p=0.005), which is commonly associated with monocytic differentiation. This was in agreement with Tonks et al., 2007, which demonstrates expressing association of inv (16) (generally associated with FAB-M4 easo) with significantly overexpressed CD200 when compared to M4 patients without inv (16). These all conduct to CD200 is expressed more with monocytic differentiated AML than in myeloid differintiated AML which is in agreement with Muhsin et al., 2017 who found CD200 expressed more in monocytic subtypes (M4-M5), and found extramedullary manifestation more with CD200 positive expression.

Table 1. The distribution of AML cases according to WHO classification

			Cases/Total %
WHO classification for AML patients:	**AML with recurrent genetic abnormalities**	AML with t(8,21)(q22;q22); RUNX1-RUNX1T1	4/104(3.8%)
		AML with inv(16)(p13.1q22); CBFB-MYH11	10/104 (9.6%)
		AML with t(9,22)(q34,q11); BCR-ABL1	5/104 (4.8%)
		AML with mutated NPM1	6/104 (5.8%)
		AML with mutated NPM1 and with t(8;21)	1/104 (1%)
	AML not otherwise specified	AML with minimal differentiation	1/104 (1%)
		AML without maturation	26/104 (25%)
		AML with maturation	20/104(19.2%)
		Acute myelomonocytic leukemia	23/104(22.1%)
		Acute monoblasic/monocytic leukemia	8/104(7.7%)
Genetic risk		Intermediate	66/101 (65.3%)
		Favorable	12/101(11.9%)
		Adverse	23/101 (22.8%)

*Some of the genetic abnormalities not performed, as; inv3, CEBPA and RUNX1.

Table 2. Treatment outcome of AML patients

Treatment outcome and follow up		Cases/Total %
Death before induction	No	91/104 (87%)
	Yes	13/104 (12.5%)
After Induction Therapy	CR1	40/91(44%)
	Death before d28	34/91(37.4%)
	Refractory	17/91(18.6%)
After Reinduction	dead	12/17 (70.6%)
	CR2	5/17(29.4%)
Relapse	yes	5/45 (11.1%)
Last follow up	Dead	62/104(59.6%)
	Alive	42/104(40.4%)

Table 3. Positive and negative expression of CD200

		Frequency (percent)
CD200 expression	**Positive**	62/104 (59.6%)
	Negative	42 (40.4%)

Table 4. Relationship between CD200 expression with age, sex and clinical data of the AML Patients

		CD200 expression		P value
		Negative	Positive	
Age		50 (20-71)	37 (19-60)	**0.002**
Sex	Male	22 (52.4%)	26 (41.9%)	0.322
	Female	20 (47.6%)	36 (58.1%)	
HM	No	37 (88.1%)	56 (90.3%)	0.753
	Yes	5 (11.9%)	6 (9.7%)	
SM	No	37 (88.1%)	56 (90.3)	0.753
	Yes	5 (11.9%)	6 (9.7%)	
LN	No	36 (85.7%)	50 (80.6%)	0.602
	Yes	6 (14.3%)	12 (19.4%)	
Gum hyper	No	41 (97.6%)	53 (85.5%)	**0.046**
	Yes	1 (2.4%)	9 (14.5%)	

** P-value ≤ 0.05 is statistically significant. NS: Non-significant. S: significant.*

Table 5. Relation of CD200 expression with laboratory findings

			CD200 expression		P value
			Negative	Positive	
CBC	Initial TLC		29.5 (1.2-351)	32.8 (1.1-328)	0.931
	Initial Hb		7.7 (4.1-13.6)	7.9 (3.7-11)	0.926
	Initial PLT		43 (3-336)	30 (1-496)	0.141
	Initial PB Blasts		68 (1-97)	60 (0-99)	0.446
BMA	Initial BM blast		75 (25-86)	73 (26-92)	0.499
	Initial Cellularity	Hypocellular	2 (4.8%)	3 (4.8%)	0.295
		Normocellular	3 (7.1%)	11 (17.7%)	
		Hypercellular	37 (88.1%)	48 (77.4%)	
FAB	M0		1 (2.4%)	0 (0.0%)	0.196
	M1		10 (23.8%)	16 (25.8%)	
	M2		14 (33.3%)	14 (22.6%)	
	M4		12 (28.6%)	28 (45.2%)	
	M5a		3 (7.1%)	4 (6.5%)	
	M5b		2 (4.8%)	0 (0.0%)	
IPT	Myeloid		20 (47.6%)	24 (38.7%)	0.331
	Myeloid with monocytic		12 (28.6%)	28 (45.2%)	
	Monocytic		5 (11.9%)	4 (6.5%)	
	Myeloid with abbarent CD7		4 (9.5%)	6 (9.7%)	
	Myeloid with abbarent CD19		1 (2.4%)	0 (0.0%)	
IPT	Monocytic		17(40.4%)	32(51.6%)	0.264
	Others		25(59.6%)	30(48.3%)	
CD34	Negative		17 (42.5%)	11 (17.7%)	**0.012**
	Positive		23 (57.5%)	51 (82.3%)	
HLA-DR	Negative		9 (22.5%)	6 (9.8%)	0.080
	Positive		31 (77.5%)	55 (90.2%)	
MPO(cyto)	Negative		6 (14.3%)	4 (6.5%)	0.309
	Positive		36 (85.7%)	58 (93.5%)	
CD13(cyto)	Negative		3 (8.6%)	0 (0%)	**0.029**
	Positive		32 (91.4%)	54 (100%)	
CD33	Negative		5 (12.2%)	5 (8.2%)	0.518
	Positive		36 (87.8%)	56 (91.8%)	
CD13	Negative		11 (27.5%)	16 (27.1%)	0.967
	Positive		29 (72.5%)	43 (72.9%)	
CD117	Negative		12 (30.8%)	13 (22.8%)	0.479
	Positive		27 (69.2%)	44 (77.2%)	
CD14	Negative		24 (64.9%)	38 (65.5%)	0.948
	Positive		13 (35.1%)	20 (34.5%)	
CD11c	Negative		14 (42.4%)	27 (54.0%)	0.372
	Positive		19 (57.6%)	23 (46.0%)	
CD64	Negative		21 (60.0%)	29 (60.4%)	0.969
	Positive		14 (40.0%)	19 (36.9%)	
Cytogenetics	Normal		8 (36.4%)	12 (35.3%)	0.053
	Abnormal		6 (27.3%)	5 (14.7%)	
	Recurrent		4 (18.2%)	16 (47.1%)	
	Complex		4 (18.2%)	1 (2.9%)	
	Wild		26 (65.0%)	48 (82.8%)	

FLT-ITD	Mutant	14 (35.0%)	10 (17.2%)	**0.045**
NPM	Wild	8 (61.5%)	20 (90.9%)	**0.036**
	Mutant	5 (38.5%)	2 (9.1%)	
t(8;21)	Negative	39 (97.5%)	54 (93.1%)	0.646
	Positive	1 (2.5%)	4 (6.9%)	
t(16;16)/inv16	Negative	40 (100.0%)	48 (82.8%)	**0.005**
	Positive	0 (0.0%)	10 (17.2%)	
t(9;22)	Negative	37 (92.5%)	59 (96.7%)	0.382
	Positive	3 (7.5%)	2 (3.3%)	
FAB	M0	1 (2.4%)	0 (0.0%)	0.196
	M1	10 (23.8%)	16 (25.8%)	
	M2	14 (33.3%)	14 (22.6%)	
	M4	12 (28.6%)	28 (45.2%)	
	M5a	3 (7.1%)	4 (6.5%)	
	M5b	2 (4.8%)	0 (0.0%)	
Genetic risk	Intermediate	23 (57.5%)	43 (70.5%)	0.168
	Favorable	4 (10.0%)	8 (13.1%)	
	Adverse	13 (32.5%)	10 (16.4%)	
WHO Classification	AML,NOS	34 (81.0%)	46 (74.2%)	0.483
	AML with recurrent genetic	8 (19.0%)	16 (25.8%)	
WHO subclassificati on	AML with maturation	9 (21.4%)	11 (17.7%)	0.101
	Acute myelomonocytic leukemia	9 (21.4%)	14 (22.6%)	
	AML with t(8;21)	1 (2.4%)	3 (4.8%)	
	AML with mutated NPM1	5 (11.9%)	1 (1.6%)	
	AML with out maturation	10 (23.8%)	16 (25.8%)	
	AML with inv(16)	0 (0.0%)	10 (16.1%)	
	Acute monoblastice/monocytic	4 (9.5%)	4 (6.5%)	
	Provsional entity: AML with BCR-ABL1	3 (7.1%)	2 (3.2%)	
	AML with minimal differentiation	1 (2.4%)	0 (0.0%)	
	AML with t(8;21) and mutated NPM1	0 (0.0%)	1 (1.6%)	

Table 6. Overall survival and its relation to CD200 expression

CD200	Mean	Std. Error	95% Confidence Interval		P value
			Lower Bound	Upper Bound	
Negative	2.832	.499	1.853	3.811	**0.007**
Positive	7.739	.868	6.037	9.441	
Overall	6.286	.671	4.970	7.601	

** P-value ≤ 0.05 is statistically significant*

Table 7. Disease free survival and its relation to CD200 expression

CD200	Estimate	Std. Error	95% Confidence Interval		P value
			Lower Bound	Upper Bound	
Negative	15.453	2.733	10.097	20.809	0.912
Positive	7.170	.303	6.576	7.764	
Overall	15.597	1.031	13.578	17.617	

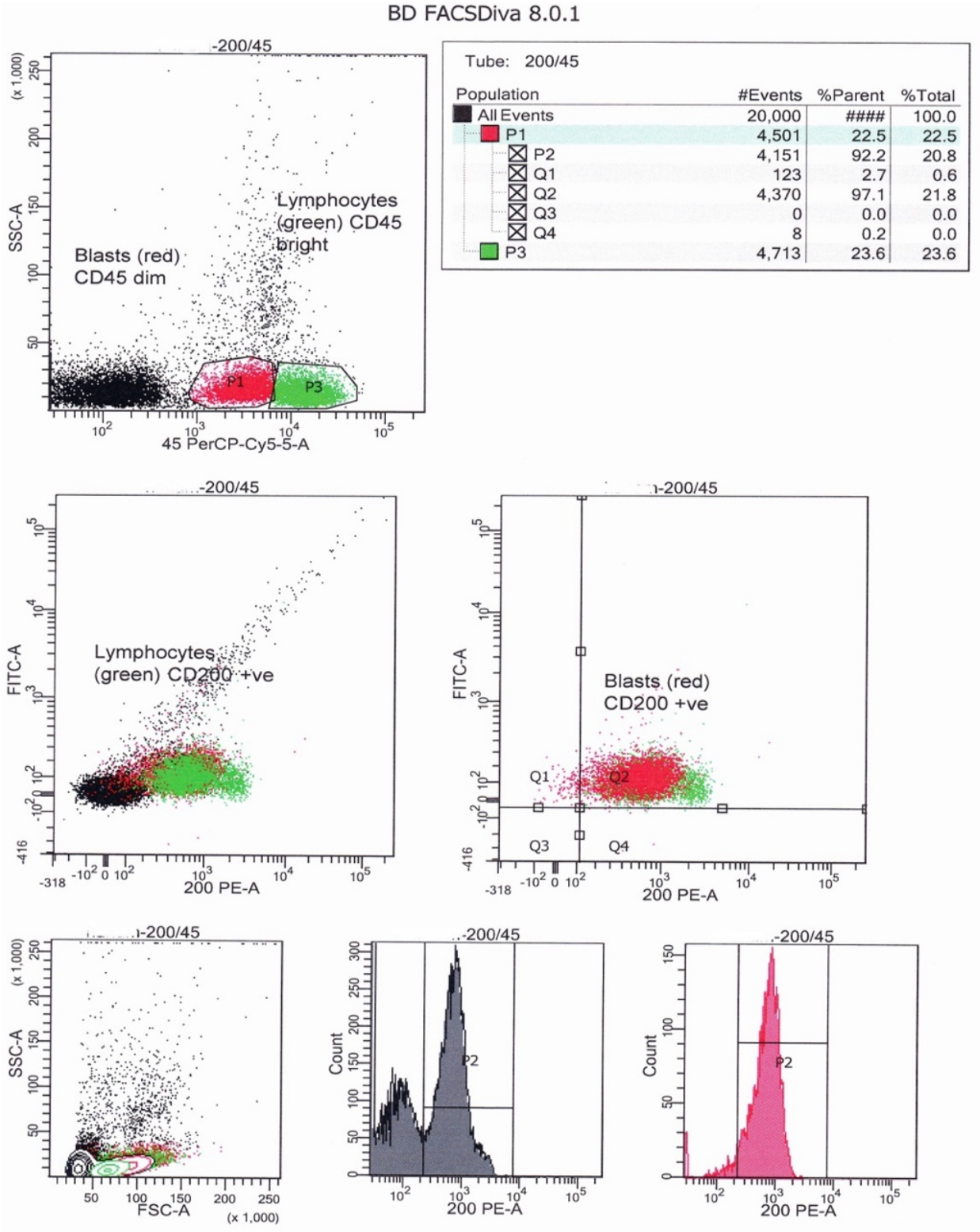

Figure 1. Expression of CD200 within CD45 dim population

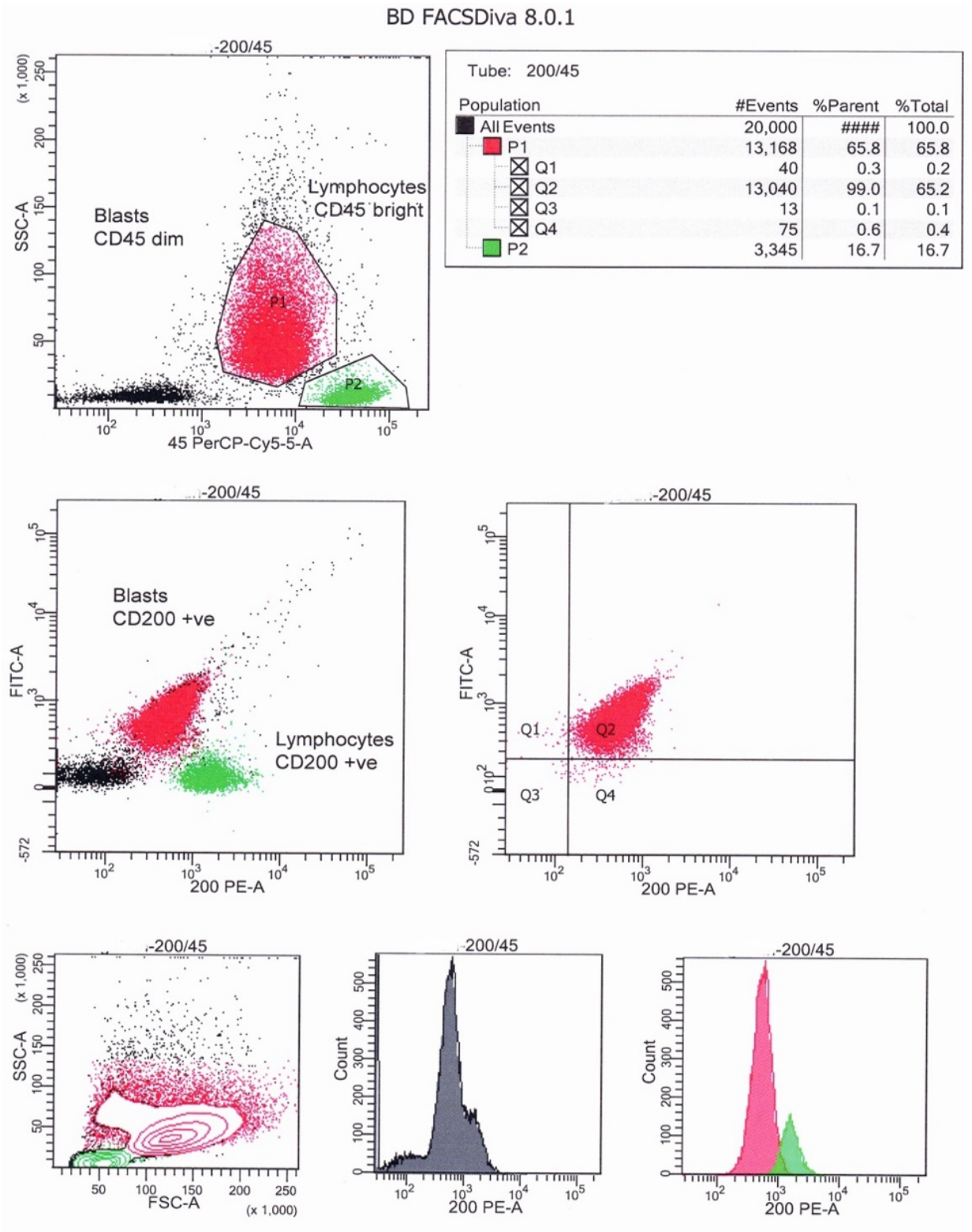

Figure 2. Expression of CD200 within CD45 dim population

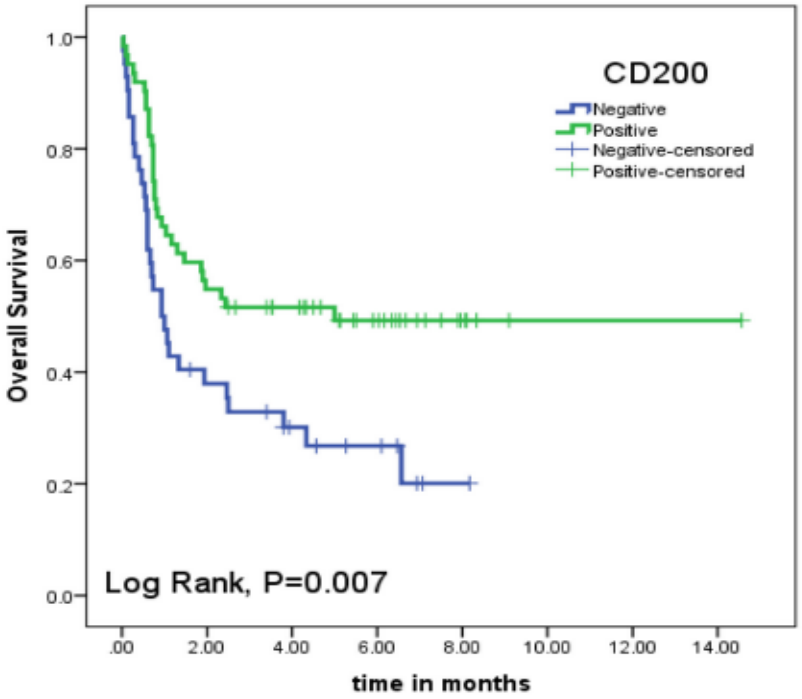

Figure 3. Relation between CD200 and overall survival

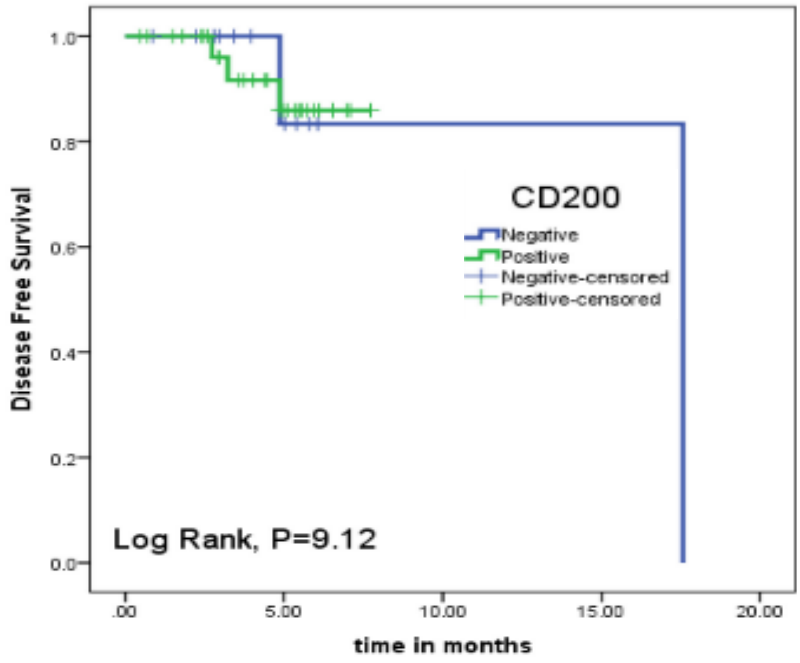

Figure 4. Relation between CD200 and disease-free survival

There was a statistically significant relation between CD200 postive expression and CD34 positive cases. This was in agreement with Tiribelli et al., 2017, Damiani et al., 2015 and Zhang et al., 2014. It could be speculated that CD200 in CD34 positive leukemic cell is normally expressed on CD34 positive leukemic progenitors, where it contributes in protection from auto-aggression by the immune system cells (Damiani et al., 2015), as the function of CD200 includes induction of immune suppression (Tonks et al., 2007). Tiribelli et al., 2017, said that a higher frequency of CD200 expression was found in CD34 positive cases,

within this group, patients with high CD200 had significantly lower OS probability compared to those with low CD200 positivity and the association of CD200 and CD34 expression implies the existence of a more stemness in cells remains to be explained. Another study was performed by Zhang et al., 2014, who concluded that the CD200 antigen expression in AML may associate with a poor prognosis.

Molecular analysis revealed that wild FLT3/ITD and wild NPM were significantly more detected in AML patients with positive CD200 patients than in AML patients with negative CD200 expression (P=0.045 and 0.036 respectively). This was equivalent to Damiani et al., 2015, where a high frequency of CD200 expression was detected in patients with wild Flt3/ITD (105/170, 62%) and in patients with wild NPM (99/145, 68%). But not with Tiribelli et al., 2017, who found no differences in CD200 expression rate compared to and FLT3/ITD mutation and NPM mutation status. That suggest that CD200 positivity is common with unfavorable genetic type.

Concerning overall survival and disease-free survival by Kaplan-Meier analysis of our data, results revealed that there was a statistically significant relation between CD200 positive expression and longer overall survival (p=0.007). While regarding the impact of CD200 expression on disease-free survival, results revealed that there was no statistically significant relation between CD200 expression and disease-free survival. In contrast to Damiani et al., 2015 and Tiribelli et al., 2017, found that CD200 positivity was associated with lower rates of complete remission and survival. Also Atfy.et al., 2015, found that the survival time in CD200 high AML patients was shown to have inferior survival compared with those CD200 low.

This conflict with this in our study may be due to the small sample size and short follow-up time (one year) while in Damiani et al., 2015 and Tiribelli et al.,2017 study; it takes 3 years of follow-up. with a large sample size. We studied CD200 as the aberrant expression on blast cells with a cutoff ≥20% and not studied as Atfy et al., 2015, who further classified the patients with Aberrant CD200 expression according to the level of CD200 expression into CD200 high and low group. CD200 positivity, per se, did not impact on DFS, but cases with high CD200 expression had a lower 3-year DFS compared to CD200-negative and low-expressing ones (Tiribelli et al., 2017).

CONCLUSION

In conclusion, CD200 was positively expressed in 59.5% of our AML patients, CD200 expression was found more in AML with monocytic differentiation compared to myeloid leukemia and in patients with gum hyperplasia and inv16 which are common presentations in AML with monocytic differentiation. there was a statistical significance between CD200 expression and CD34 positive cases. CD200 expression was significantly related to wild FLT3/ITD and wild NPM.

Previous studies suggested that blocking CD200 by the use of antibody treatment leads to inhibition and suppression of the interaction between CD200 and CD200R. That will activate the patient immune cells, making CD200 a potential therapeutic target for CD200-positive AML. Blocking and inhibition of CD200 on AML blasts by inhibiting its interaction with CD200R lead to restoring of adaptive and innate immune response for the destruction of tumor cells.

Immunophenotypic evaluation of CD200 should be incorporated in the initial diagnostic workup of AML for new targeted therapy involving CD200 pathway. Inter-laboratory trials should be planned to standardize analysis, make results comparable and find the appropriate cut-off level of expression.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

All eligible individuals agreed to voluntary participation and signed an informed consent form.

AUTHOR CONTRIBUTIONS

Participated in research design: Naglaa M. Hassan. Sample collection and clinical evaluations: Ahmed Magdi Rabea. Conducted experiments: Mohamd Mostafa. Analysis of data: Rania Soliman. Literature search: Mohamd Mostafa. Wrote or contributed to the writing of the manuscript: Reem Nabil. Revision of the article: Hala Mohammad Farawela. All authors read and approved the final manuscript.

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