

CD56 and CD19 Antigens Expression in Acute Myeloid Leukemia Identifies Patients with Adverse Prognosis in Egypt

Running head: CD56 and CD19 antigens and Acute Myeloid Leukemia prognosis

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Abstract: CD56 was firstly described as a marker of natural killer cells and has been found expressed in several neoplasms including acute myeloid leukemias (AML), the presence of CD56 antigen on blast cells may influence complete remission and survival. CD19 is a B-lymphocyte marker, whose expression is associated with pediatric AML-M2 and the t(8;21) translocation. The biological and clinical significance of CD19 expression in AML is not clear. **Patients and Methods:** fifty de-novo AML were included, bone marrow aspirate subjected to immunophenotyping for lymphoid marker CD 19 and CD56, and cytogenetic study (karyotyping and FISH) and results were correlated with clinical outcome. **Results:** Fifty patients were included of which, 22 were male and 28 were female, with a median age of 40 years (16-75). There is a significant correlation between CD56 expression and cytogenetic abnormalities associated with unfavorable prognosis ($P = 0.001$), while the correlation between CD19 expression and cytogenetic analysis was not significant ($p=0.06$). CD56& CD19 expression did not influence CR rate ($P = 0.51$, $p=0.08$; respectively). Expression of CD56& CD19 had adverse effect on DFS ($p=0.03$ and $p<0.00$; respectively), and on OS ($p=0.001$ and $p=0.001$; respectively). **Conclusion:** CD56 and CD19 expression may identify acute myeloid leukemia patients with adverse prognosis.

Keywords: Acute Myeloid Leukemia; Immunophenotyping; CD56; CD19, prognosis.

1. Introduction

Acute myeloid leukemia (AML) could be considered as a heterogeneous group of disorders which often present with different morphological, immunophenotypic and cytogenetic patterns (1–3). Identification of these characteristics may be useful for a better prognostic evaluation and for a more appropriate therapeutic approach.

Occurrence of aberrant phenotype has been reported in acute leukemias with varying frequency though its prognostic importance remains controversial (1).

CD56 antigen, a 200–220 kDa cell surface glycoprotein, identified as an isoform of the neural adhesion molecules (NCAM)(4-6), it was firstly described as a marker of natural killer cells and subsequently, has also been found expressed in several lympho–hematopoietic neoplasms including acute myeloid leukemia (AML)(7-10). In fact, it has been previously reported that in AML patients with t(8;21) (q22;q22), generally considered at lower risk of relapse, the presence of CD56 antigen on blast cells may influence complete remission (CR) duration and survival (11), suggesting that CD56 expression could be useful in stratifying therapeutic approaches for this subtype of AML.(11,12).

CD19 is a phosphoglycoprotein lymphoid antigen which expressed normally on follicular dendritic cells& B cells; it is commonly expressed in AML-M2 (13).

In order to better clarify the prognostic role of CD56& CD19 expression in AML cells, we evaluated the presence of these antigens on leukemic cells of fifty newly diagnosed AML patients and results were correlated with the clinical outcome.

2. Patients & Method

Fifty newly diagnosed adult AML cases presenting to Medical oncology, clinical pathology departments, Zagazig University, and Hematology unite, Internal medicine department, Mansoura University, Egypt,(between may, 2013 and may, 2014) were included in this study.

Before starting chemotherapy, adequate immunophenotype studies and, in the majority of patients, cytogenetic analyses were performed.

Complete blood count (CBC) was done using automated cell counter; Sysmex SF 3000(Roche-Diagnostics,Manheim, Germany). Bone marrow aspirates were examined for the presence of blast cells and the diagnosis of each leukemias subtype was established according to morphological, cytochemical and immunological criteria according to the French-American-British (FAB) and World Health Organization (WHO) classifications.

3. Conventional karyotyping

Culture: The BM cells(on heparin) were cultured in a medium {RPMI 1640 supplemented with fetal calf serum (Gibco ,USA), L-glutamine (Gibco BRL) penicillin and

streptomycin, (Biochrom) incubated at 37°C in a strict sterile condition. three cultures tubes were used for each patient and should be kept for 24, 48 and 72 hours.

Harvesting: using colcemid 50 µl {10 µg/ml (Gibco BRL)} for 20 minutes then 10 ml hypotonic solution were added (potassium chloride (5.59g/L)) for 30 minutes at 37°C
Fixation: 10 ml fixative solution (absolute methanol (3 parts) mixed with glacial acetic acid (1 part)) were added and left for 10 minutes this step was repeated twice then the pellet was kept at fridge overnight.
Chromosome Spreading: 3-4 drops of cells suspended in fixative were allowed to fall on a cold pre-cleaned glass slide from a height of about 40-60 cm to obtain good cell spread
G-Banding: after aging of cells for 48 hours at room temperature banding were done using trypsin solution (Gibco, USA) (Seabright, 1971).

At least 20 metaphases were examined; an automated karyotyping system was used for analysis (IMSTAR, France) Karyotyping was done according to the International System for Human Cytogenetic Nomenclature (ISCN, 2005, Karger).

Fluorescence in situ hybridization

FISH assays were performed by following the probe manufacturer instructions {t(8;21) (q22;q22) RUNX1/RUNX1T1, inv(16)(p13.1q22)CBFB/MYH11, and t(15;17)(q22;q12)PML/RARA dual fusion color aquarius, Cytocell., UK}

The slides were analyzed using an epifluorescence microscope (Olympus, BX63) and a computerized image analyzer cytovision software (Applied Imaging, Genetix Europe) A minimum of 200 cells per specimen/probe were scored

For Immunophenotyping, bone marrow cells were stained with various combinations of fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chlorophyll protein (PerCP) -labeled monoclonal antibodies against the following antigens: CD3, CD5, CD13, CD14, CD19, CD20, CD34, CD41 and CD45 (BD, Biosciences, San Jose, CA), CD7, CD22, CD33, CD64, glycophorin A and CD10 (Dako), other antibodies were used to identify cytoplasmic antigens as a triple color MPO, CD3, CD79a (BD) and nuclear terminal deoxynucleotidyl transferase (TdT) (Dako).

Cell surface immunophenotyping was performed for CD56 and CD19 on blast cells in acute myeloid leukemia. CD56 (PerCP) CD19 (FITC) were obtained from R&D system (Minneapolis, USA). Gating on myoblasts was based on CD45 versus side scatter analysis and a co-expression of CD56 and CD19 on myeloblast was detected.

Statistical Analysis

Disease free survival (DFS) was calculated from time of complete remission (CR) till relapse or last follow up to, and overall survival (OS) was calculated from diagnosis till death or last follow up. Survival was plotted with Kaplan-

Meier curves, and the data for the various groups were compared with a log-rank test. Multivariate analysis was performed by Cox model after the proportional hazard assumption was checked. P value less than 0.05 was considered to be statistically significant.

4. Results

The baseline characteristics of the 50 patients who were included in the study are summarized in Table (1). Fifty de-novo AML patients were included of which, 22 were male and 28 were female, with a median age of 40 years (16-75). All patients under 65 years underwent the same chemotherapy protocol consisting of first-line induction and consolidation regimens. First-line induction regimen comprised a 7-day continuous infusion of standard-dose cytosine arabinoside (100 mg/m²) and doxorubicin (30 mg/m²) for 3 days (3-7 protocol). The consolidation treatment included the administration of high-dose cytosine arabinoside (1g/m², daily, days 1-5). Patients more than 65 years old had reduced drug doses and 3 out of 50 evaluable patients underwent allogeneic bone marrow transplantation within 6 months after achieving CR.

Thirteen patients presented with pallor, 30 with fever, 16 with purpura and 3 patients presented with organomegally. According to FAB criteria, patients were classified into 5 as M1, 14 as M2, 6 as M3, 9 as M4, 11 as M5 and 2 as M6 and 3 as Mixed Phenotype Acute Leukemia (MPAL).

Correlation between CD56 & CD19 expression with immunophenotype and cytogenetic analysis:

CD56 was expressed on 21/50 cases (38%), when correlated with FAB criteria, CD56 expression, was detected in 3/5 (60%) M1, 6/14 M2, 0/6 M3, 4/9 (44%) M4, 6/11 (55%) M5, 0/2 M6 and 2/3 (67%) in mixed lineage acute leukemia (p=0.2).

Cytogenetic analysis was available only in 40 out of 50 patients, 15 of whom were CD56+ and 25 were CD56-, the cytogenetic studies were failed culture in 10 patients, CD56 expression was positive in 11/16 (69%) unfavorable cytogenetic, and negative in all favorable cytogenetic cases, so CD56 was significantly expressed in patients with unfavorable cytogenetic abnormalities (P = 0.001).

CD19 was expressed on 17/50 (34%) cases, 2 as FAB M1, 5 cases were FAB M2, one case was FAB M3, 3 as M4, 4 as M5 and 2 cases for MPAL (p=0.67).

CD19 was positive in 9/16 (56%) of unfavorable cytogenetics, and positive only in 1/7 (19%) of favorable cytogenetics, so there is no significant correlation between CD19 expression and cytogenetic analysis (p=0.06).

Correlation of CD56 & CD19 expression with clinical outcome

To address the hypothesis of whether CD56 & CD19 expression could represent as adverse prognostic factors in AML, their presence were correlated with clinical response. The response was evaluated after induction therapy. The

overall CR rate in our series of patients was 74%. However, among CD56- patients, CR was achieved in 21 / 29 patients (72.4%), and in 16 out of 21 (76.2%) of CD56+ cases (P = 0.51). Overall, CD56 positivity did not influence CR rate.

Also in CD 19 + cases, CR was achieved in 10 patients (58.8%), while in CD19- cases CR was achieved in 27 (81.8%) patients (p=0.08).

After a median follow up 15 months, Median DFS was not reached, while mean DFS of all patients was 13.9 months. In CD56+ cases, mean DFS was 11 vs. 16 months in CD56- cases. Also in CD19 + cases, mean DFS was 8 vs. 16 months in CD19- cases. So expression of CD 56, CD19 have adverse effect on DFS (p=0.003 and p< 0.001; respectively) (Figure 1, 2).

Death occurred in 26% of the patients (13/50), median OS was not reached, while mean OS was 22.9 months. Mean OS of patients with CD56+ was 15 vs.27 months in CD56 - cases (p=0.001). In CD19 + cases, the mean OS was 10.9 months while it was 26.4 months in CD19- cases (p=0.001), so patients with expression of CD 56 ,CD19 have worse OS (Figure 3, 4).

Co-expression of both CD56 and CD19 marker was detected in 11cases (22%), 9 of them had unfavorable cytogenetics; the other 2 had failed cytogenetic (p< 0.001) also Co-expression of both CD56 and CD19 genes had a significant adverse effect on DFS and OS (p <0.001, and p< 0.001; respectively; figure 5, 6).

Impact of prognostic factors on Disease-free survival and Over- all survival by univariate and multivariate analysis was shown in table (2).

Purpura organomegally	16	32%
Response to treatment:		
CR	37	74%
Not CR	13	26%

Table 2: Impact of prognostic factors on Disease-free survival and Over- all survival.

DFS				
Prognostic Factor	Univariate analysis	Multivariate analysis		
		HR	95% CI	P
Age	0.23	0.2	0.4-2.4	0.19
WBCs	0.59	0.7	0.3-2	0.6
Cytogenetics risk	0.11	0.4	0.17-1.4	0.2
CD 56	0.03	0.26	0.09-0.68	0.006
CD19	<0.001	0.14	0.0-0.41	<0.001
Co expression of CD56 and CD19	<0.001	0.06	0.015-0.255	<0.001
OS				
Prognostic Factor	Univariate analysis	Multivariate analysis		
		HR	95% CI	P
AGE	0.6	3.3	0.2-53.7	0.3
WBCs	0.4	1.18	0.3-3.7	0.7
Response to treatment	0.15	2.5	0.7-6.9	0.15
Cytogenetics risk	0.18	0.2	0.4-2.8	0.2
CD56	<0.001	0.4	0.05-3.4	0.2
CD19	<0.001	0.26	0.07-2.8	0.2
Co expression of CD56 and CD19	<0.001	0.17	0.05-0.5	0.003

Table 1: Shows patients characteristics:

Patients characteristics	NO	Percentage
Age:		
≤ 60 years	45	90%
> 60 years	5	10%
Sex:		
Male	22	44%
Female	28	56%
FAB Classification:		
M1	5	10%
M2	14	28%
M3	6	12%
M4	9	18%
M5	11	22%
M6	2	4%
Mixed lineage	3	6%
Cytogenetic:		
Favorable	7	14%
Intermediate	17	34%
unfavorable	16	32%
failed	10	20%
Presentation		
Pallor	13	26%
Fever	30	60%

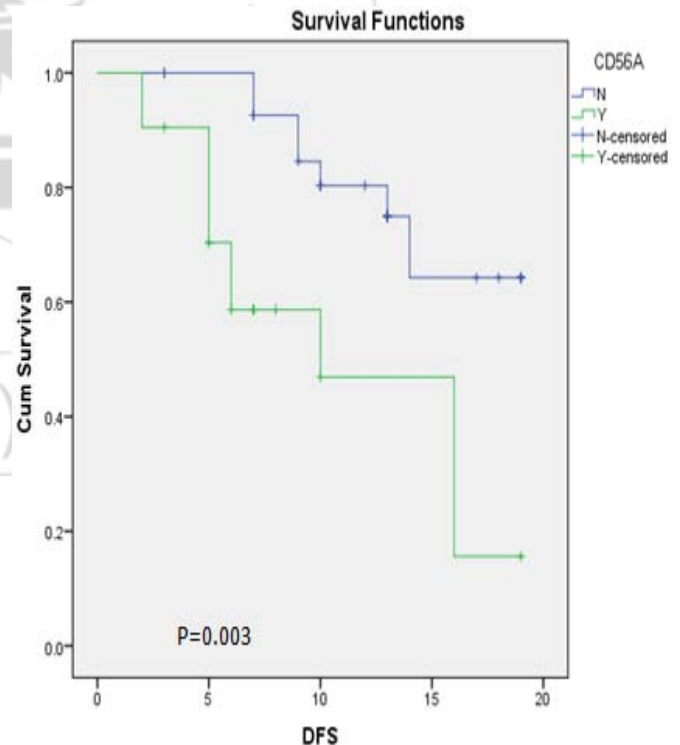


Figure 1: Correlation between CD56 expression and DFS

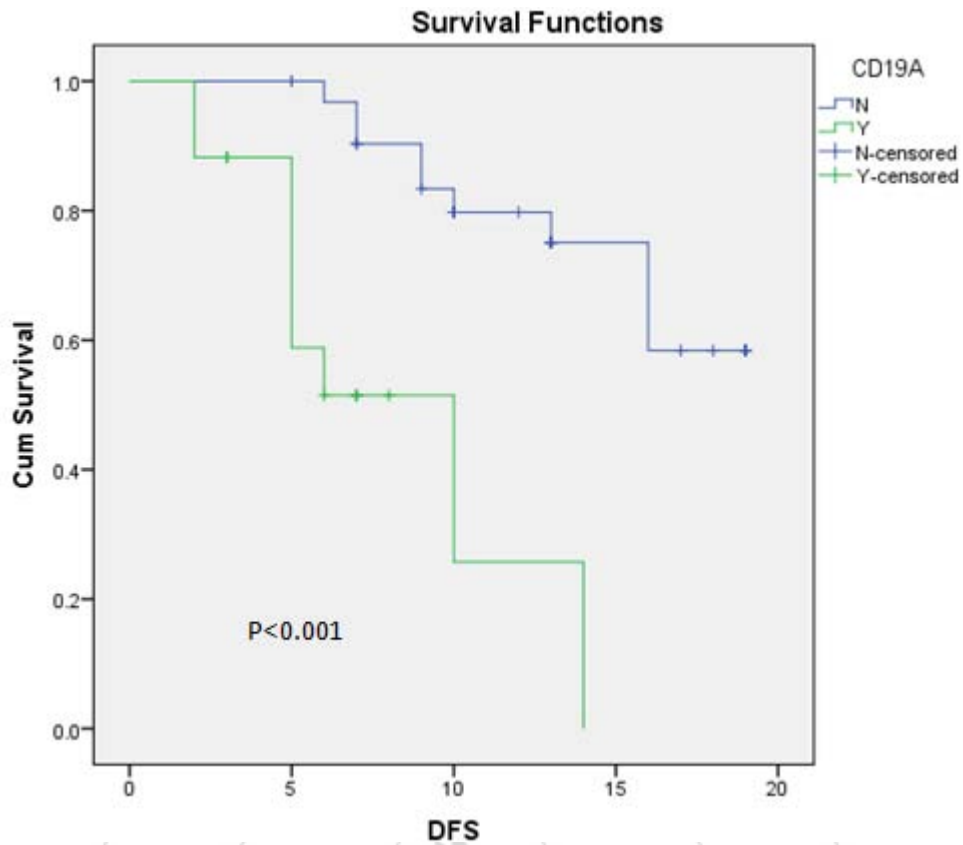


Figure 2: correlation between CD19 expression and DFS

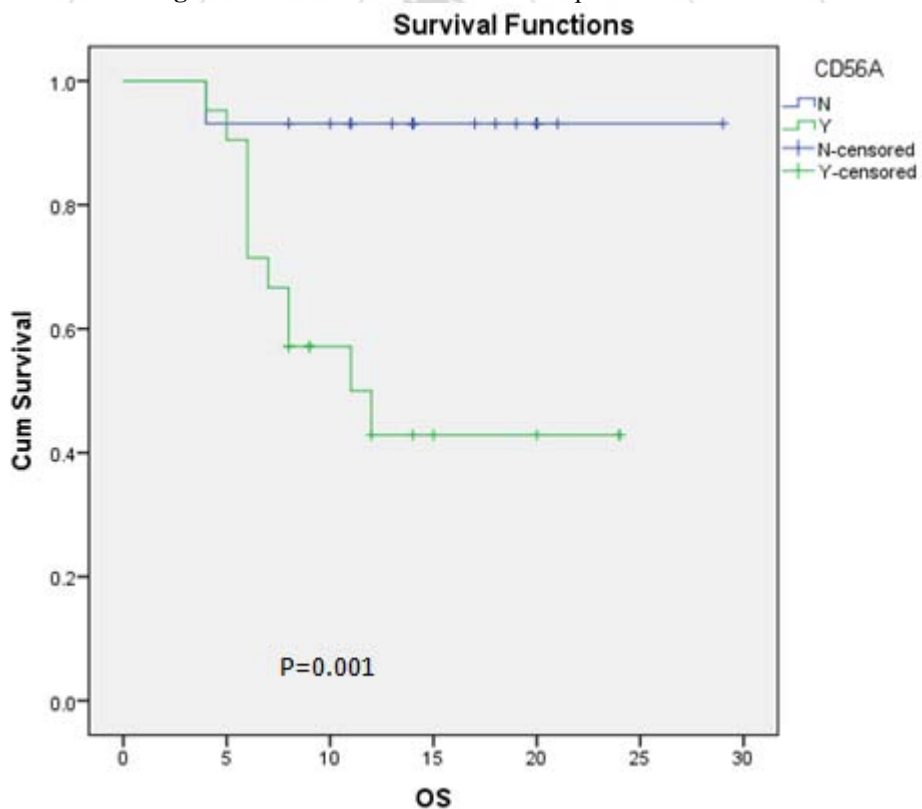


Figure 3: correlation between CD56 expression and OS

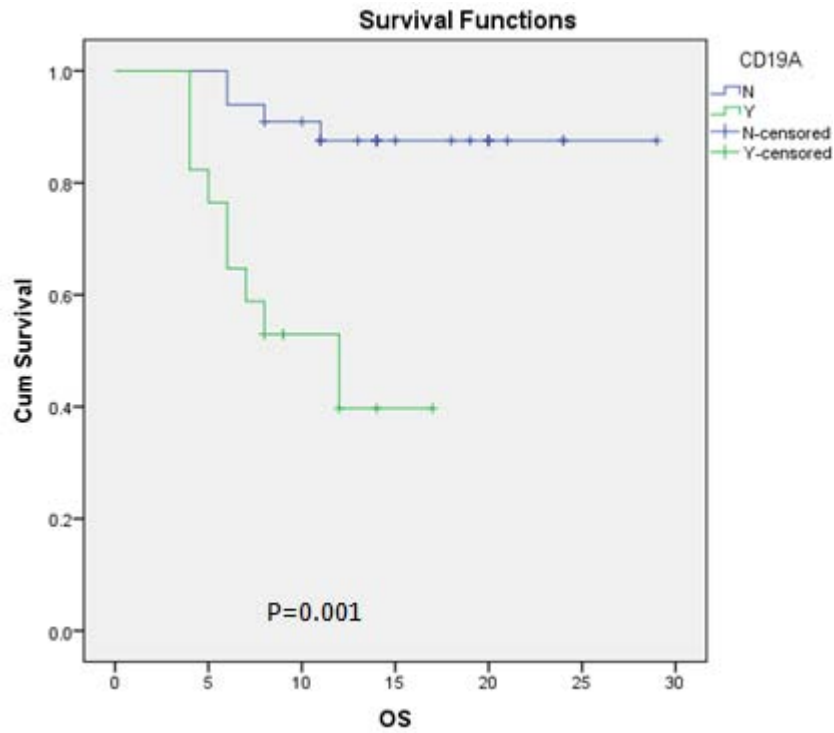


Figure 4: correlation between CD19 expression and overall survival

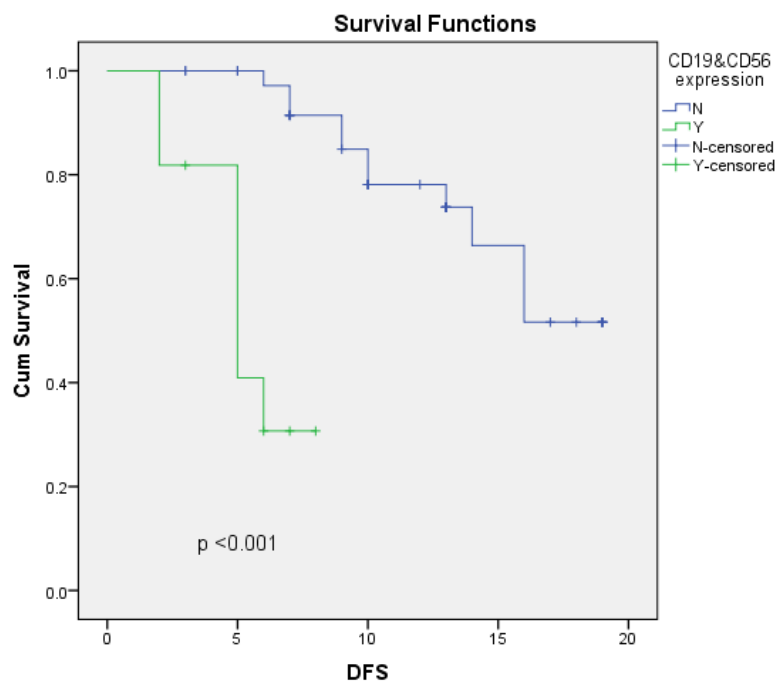


Figure 5: correlation between co expression of both CD56& CD19 and DFS

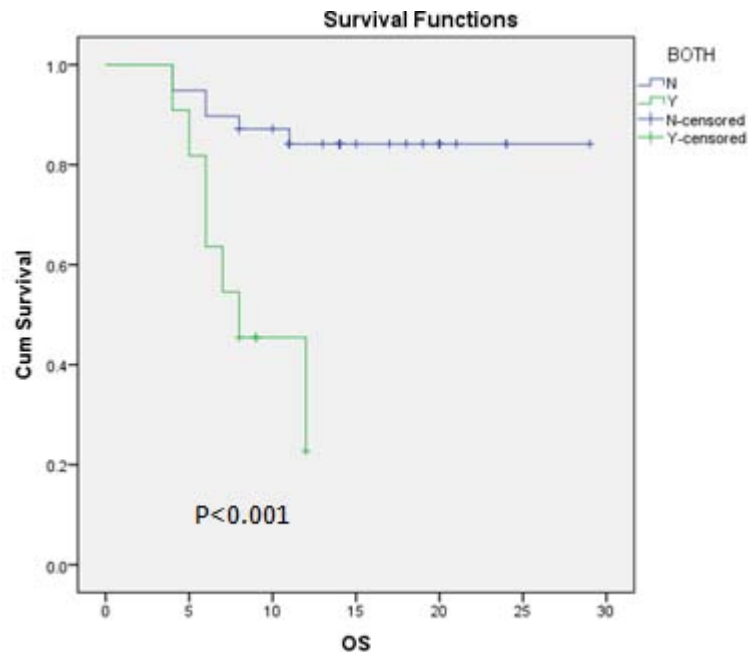


Figure 6: correlation between co expression of both CD56& CD19 and overall survival

5. Discussion

Several studies reported the correlation between myeloid blast cell immunophenotypic, morphology, cytogenetic pattern and patient's clinical outcome (14–16). For example, lymphoid-associated antigens such as CD19 has been considered an adverse prognostic factor for AML patients (17-19). Recently, many studies have addressed the role of CD56 expression in hematological malignancies. In fact, this antigen is an isoform of the neural adhesion molecules (NCAM), has been recorded in several myeloproliferative disorders including acute leukemias (20–25). In this latter setting, CD56 expression was frequently associated with a poorer outcome (25), in AML patients with t(8;21) (q22;q22) and in APL cases, which are considered AML with good prognosis, the presence of CD56 antigen on the membrane of leukemic blasts led to a significant reduction of CR and overall survival duration (25–27).

In our study CD56 was expressed in 38 % of cases, and seen in all FAB types except M3 and M6. However highest frequency was seen in MPAL type, while Raspadori D et al (28), reported that CD56 antigen was detected in only 24% of cases and CD56 antigen was rarely expressed in M0 patients, but was more frequently expressed in M2 and M5 cases.

Also in our study CD 19 was expressed in 34 % of cases and not limited to M2 but it was seen in M1, M3 and M4 and showed highest expression in MPAL (66.6%). While, in a study from Taiwan (29), CD19 expression was only observed in AML-M2 (5/36, 14%), Zheng J et al and Bahia DM et al (30, 31) also found that CD19 was expressed at highest rate in AML M2.

In concordance with our results, Jha R et al (32) observed that CD 19 was not limited to M2 but showed highest expression in M0 (15.6%).

Among 6 cases of FAB M3 morphology with t(15;17) included in our study, 1 case had CD19+ antigen but no cases expressed CD56, and this also found in EL-SISSY A et al (33), who reported that among 6 cases of FAB M3 morphology included in his study, 3 cases expressed lymphatic antigens, but in contrast to our study, Wang et al (34), reported that no co expression of lymphoid antigens was detected among 7 M3 cases with t(15;17).

Cytogenetic analysis was available only in 40 out of 50 of our patients, and there was a significant correlation between CD56, CD19 expression and cytogenetic abnormalities associated with unfavorable prognosis ($P= 0.001$, $p=0.006$ respectively), and this result was matched with those reported by Raspadori D et al.(28), where a cytogenetic analysis was evaluable only in 132 out of 152 patients, a significant correlation between CD56 expression and cytogenetic abnormalities associated with unfavorable prognosis was documented both in univariate and multivariate analysis ($P = 0.008$ and 0.025 , respectively).

The clinical relevance of lymphoid antigen expression (LY+) in AML has been highly controversial. Some studies have reported LY+ AML to be associated with the poor prognosis (35- 37), but some reported it to be associated with favorable prognosis (38), whereas other suggest it to be of no prognostic value (30).

In our study, CD56 positivity did not influence CR rate ($p=0.51$). This may be due to small sample size. In CD 19 + cases, CR was achieved in 58.8% of patients, while in CD19- cases CR was achieved in 81.8% of patients ($p=0.08$), and this result was matched with those reported by Jiang NG et al(39), where 91 cases of AML were included, and they found 14 cases of AML only expressed CD19, 10 cases expressed CD56, also they reported that CR ratio and DFS were lower in patients expressed both lymphatic markers, DFS of CD56+ AML patients was lower, but CR ratio had no significant difference compared with CD56- patients.

In our study, patients with expression of CD 56 and CD19 had worse OS and DFS ($p=0.001$; $p=0.001$ and $p=0.03$; $p=0.00$; respectively).

Co-expression of both CD56 and CD19 which was detected in (22%) of our cases had have adverse effect on DFS and OS by univariate and multivariate analysis.

In adverse to our results, Noriyoshi Iriyama et al (40), who investigated 144 patients with AML with $t(8;21)$. CD19 expression was (36%), and CD56 expression was (65%). CD19 expression is significantly correlated with improved prognosis in his study population, probably because the CD56-negative population frequently demonstrates CD19 positivity.

In conclusion, our data suggest that CD56, CD19 antigenic expression in AML patients is more frequently associated with unfavorable cytogenetic abnormalities. Moreover, they correlate with a reduced probability of achieving CR and with a shorter survival, and therefore, the presence of CD56, CD19 antigens on myeloid blasts should be regularly assessed in AML patients at diagnosis, and should be taken into consideration in designing future therapeutic strategies based on patient-specific risk factors.

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