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Journal of Bioscience and Applied Research

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CD47 Expression in Egyptian Patients with Acute Myeloid Leukemia

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Abstract

Background: Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disease defined by the proliferation and accumulation in the bone marrow and blood of immature hematopoietic cells. CD47 -Signal Regulatory Protein α (Sirpα) Regulate the negative regulation of phagocytosis, an inhibitory receptor. CD47 overexpression in AML is overexpressed on peripheral blasts and stem cells with leukemia and correlates inversely with survival. **Aim:** Estimation of CD47 gene expression in Egyptian AML patients for evaluation of its role in the pathogenesis of the disease and as a prognostic marker related to overall survival (OS). **Methods:** The 55 patients with AML in addition to 21 healthy individuals as a control group were included in the current study. Blood samples from control group and patients were subjected to positive magnetic selection of CD34+ cells of leukemic, and then using flowcytometry the CD47 expression on these cells was estimated. **Results:** Statistically significant difference ($P < 0.0001$) between two groups AML patients and control group in Hemoglobin (g/dL), WBCs (10^3/µL) and PLTs count (10^3/µL). The results on incidences of CD47 differ significantly among FAP type in AML patients. The mean percent of CD47 were increased with FAP type excretion, 38.6 ± 39.5 at M1, 51.1±34.9 at M2, 60.2±51.9 at M3 while, those showed decrease 33.1±22.6 with M4 and 43.2±31.4 with M5. There was an inverse correlation between concentrations of CD47 gene expression and general survival, with enhanced expression also connected with worse general survival ($P < 0.0001$). **Conclusion:** The results obtained by this study provide additional evidence of the role of CD47 gene as a predictive factor impact on prognosis and OS.

Keywords: AML; CD47; Marker; prognosis.

1.INTRODUCTION

In cancer, division and growth of cells are out of control to form lumps or masses of tissue called tumors. Cancer may also move to distant parts of the body through the blood or lymph systems and destroy healthy tissues **(Rahman and Mohammed, 2015).** The lymphatic system may also be divided into five sections primarily based on size and functionality: The lymphatic capillaries, lymphatic accumulating vessels, lymph nodes, lymphatic trunks and ducts **(Rahman and Mohammed, 2015)**. Abnormal proliferation of blood cells in the bone marrow and blood-forming organs leads to a malignant situation many times referred to as leukemia, which may also be categorized based on the pace of progression **(Kondo, 2010).** The beginning of leukemia may be sudden (acute) or slow and gradual (chronic) **(Kondo, 2010).** Malignancy involving myeloid cells, granulocytes (neutrophils, basophils, and eosinophils) and monocytes (macrophages) lead to myeloid leukemia whereas that involving T and B lymphocytes give rise to lymphocytic leukemia **(Haouas et al., 2010).** AML is a malignant bone marrow disorder in which early hematopoietic precursors are detained. Most AML subtypes are differentiated by the existence of more than 20 percent blasts in the bone marrow from other associated blood disorders **(Döhner et al., 2015).** Occasionally spread may occur to the brain, skin, or gums. As acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated **(Döhner et al., 2015).**

Many CD genes provide instructions for making proteins that are found on the surface of white blood cells (leukocytes) at various stages of their development **(Fabryova and Simon, 2009)**. CD47, a SIRPα ligand, has been described as an important anti-phagocytic signal expressed on tumor cells **(McCracken et al., 2015)**. Binding of CD47 to sign regulatory protein alpha (SIRPα), an inhibitory receptor, negatively regulates phagocytosis **(Galli et al., 2015)**. Therefore, the blockade of CD47 is attracting increasing attention as a conceivable high-quality therapeutic approach against most cancers **(Chao et al., 2012)**. The preceding observations elevate the possibility that accelerated expression of CD47 might have a function in the pathogenesis of AML; consequently, in the present learn about CD47 gene and protein expression were carried out in AML patients to evaluate their importance and relate it to universal survival. Hence, in the present find out about CD47 gene and protein expression had been performed in AML sufferers to consider their significance and relate it to universal survival.

2.SUBJECTS AND METHODS

2.1. Subjects

The study included all patients who were diagnosed to be at risk for leukemia in Mansoura University Hospital, Mansoura, Egypt during the period from 2016 to 2018. We excluded patients who met any of the following criteria: Philadelphia-positive leukemia, infantile leukemia, mixed lineage leukemia and Burkitt-type leukemia were excluded from the research because of the no similar type of treatment protocol applied. This research has been endorsed by the Mansoura University Medical Research Ethics Committee, Mansoura City, Egypt. Patients acquired written informed consent prior to involvement in the research. This study was included 55 patients with newly diagnosed Acute Myeloid Leukemia, and 21 healthy individuals as control group with matching age and sex were admitted to Mansoura University Hospital. Patients with hereditary or systemic disorders were excluded. The diagnosis was based on cytological, immunophenotypic and cytogenetic criteria.

2.2. Sampling:

Blood samples: blood from vein puncture were collected and divided into two tubes; first that contained dipotassium ethylene diamine tetra-acetic acid (K2EDTA) for complete blood cell counts (CBC). CBC was determined in each sample within 2 hours of collection. Serum was prepared by centrifuging by CENCOM® bench centrifuge (Analytika, Athens, Greece) collected blood at 4000 r.p.m. for 10 minutes, aliquoted and stored at -20°C until biochemical analysis. The clear serum was aspirated by Pasteur pipette and received in dry sterile sample tube, then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

Bone marrow samples: Bone marrow aspiration was done under complete aseptic conditions at time of diagnosis: small part of aspirate was used for spreading smears to be examined by Leishman and MPO stains, 1 ml was dispended into sterile tube containing K-EDTA to be used for the flow cytometry for immunophenotyping detection.

2.3. Methods

Complete blood count (CBC):

CBC was determined using the electronic counter (CELL-DYN 3700, Abbott, Canada) to determine HB, HCT, erythrocytes, RBCS: mean corpuscle volume (MCV), mean corpuscle HB content in RBCs (MCH).

Immunophenotyping (IPT) for surface CD47:

Bone marrow aspiration smears: for morphological diagnosis of AML. Cytochemical analysis (Sigma Diagnostics, St. Lowis, Missouri, USA) of air-dried peripheral blood and/or bone marrow smears: It is important in distinguishing AML from ALL and in sub-classifying AML. Immunophenotyping of Leukemia blast: it had an important role in distinguishing between ALL and AML and AML sub-classifying AML using, (COULTER EPICS XL™ Flow cytometer coulter electronics, Florida, USA).

Flowcytometry used to be done on the positively selected CD34+ cells via surface monoclonal antibodies directed towards human CD47 (FACS Calibur, BD). The goal population used to be CD34+ - CD47+ cells which were once analyzed on the Cell Quest program. Positivity with go with the flow cytometry was defined as an expression in at least 20% of cells in the gated populations of interest, in contrast to interior poor manipulate cells.

2.4. Statistical Analysis:

Quantitative data were presented as a minimum, maximum, mean, median and standard deviation (SD) values. Data showed a non-parametric distribution and so the Mann-Whitney U test was used for comparisons among groups. This test is the nonparametric alternative to Student's t-test. Qualitative data were presented as frequencies and percentages. Chi-square (x^2) test was used for studying the comparisons and associations between different qualitative variables. Spearman's correlation coefficient was used to determine significant correlations between the expression of CD47 and other different variables. Kaplan-Meier survival curve was constructed for survival analysis. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 24 for Microsoft Windows, SPSS Inc. and considered statistically significant at a two-sided $P < 0.05$.

3.RESULTS

3.1. Baseline characteristics of included patients

In the present study, blood samples from 55 patients with AML (32 males and 23 females) were collected from the oncology center, Mansoura University Hospitals, Mansoura, Egypt. The included AML patients had a mean age ± SD (standard division): 45.8 ± 15.1 years aged between 19 -71 years. In addition, blood samples from 21 healthy

individuals, 10 males, and 11 females, aged between 20 - 63 years with mean age: 39.9 ± 12.8 years as a control group included in the present study; **Table 1.**

3.2. Hematological parameters.

Table (1) shown the mean level of Hemoglobin (Hgb) (g/dL) in AML patients and healthy were included in the present study. There was a significant difference ($p < 0.0001$) between the two groups. The mean number of WBCs ($10^3/\mu L$) in AML patients and healthy were included in the present study. There was a significant difference ($p < 0.0001$) between the two groups. The mean count of PLTs ($10^3/\mu L$) in AML patients and healthy were included in the present study. There was a significant difference ($p < 0.0001$) between the two groups.

Table 1: Characteristics of AML patients and healthy control groups

Characteristics	Control (No = 21)	AML patients (No = 55)	P value
Gender			
Male (no., %)	10 (47.6%)	32 (58.2%)	-
Female (no., %)	11 (52.4%)	23 (41.8%)	
Age (years)			
Mean ± SD	39.9 ± 12.8	45.8 ± 15.1	*P* > 0.05
(range)	(20 – 63)	(19 – 71)	
Hb (g/dl)			
Mean ± SD	11.5±1.3	8.2 ± 2.1	*P* < 0.0001
(range)	(9.7 – 13.8)	(3.8 – 16.2)	
WBCs x 10^3/cmm			
Mean ± SD	6.2 ± 1.5	56.9 ± 16.3	*P* < 0.0001
(range)	(4.3– 9.7)	(8.4 – 280.0)	
Platelets x 10^3/cmm			
Mean ± SD	236.0±43.0	42.8±18.3	*P < 0.0001*
(range)	(183.0– 320.0)	(55.0– 161.0)	
B.M. blasts (%)			
Mean ± SD	-	68.1±20.5	-
(range)		(26.0– 100.0)	
FAP type			
M1 (no. , %)	-	10 (18.2%)	-
M2 (no. , %)	-	11 (20.0%)	
M3 (no. , %)	-	3 (3.6%)	
M4 (no. , %)	-	20 (36.4%)	
M5 (no. , %)		12 (21.8%)	

AML: patients with Acute Myeloid Leukemia, **SD:** standard division, **B.M.:** bone marrow and **FAP:** Familial adenomatous polyposis

3.3. Incidences of CD47 among FAP type in AML patients

Our results on incidences of CD47 differ significantly among FAP type in AML patients. Flow cytometric analysis of CD47 expression revealed that the mean percent of CD47 were increased with FAP type excretion, 38.6 ± 39.5 at M1, 51.1±34.9 at M2, 60.2±51.9 at M3 while those showed decrease 33.1±22.6 with M4 and 43.2±31.4 with M5; **Table 2.**

Table 2: Mean percent of CD47 gene according to FAP type (%) in AML patients

FAB type	Mean ± SD (%)	Minimum	Maximum
M1	38.6 ± 39.5	2.3	99.5
M2	51.1±34.9	3.1	97.0
M3	60.2±51.9	23.5	97.0
M4	33.1±22.6	8.4	86.8
M5	43.2±31.4	4.4	96.0
P value	P < 0.01		

Frequency of Outcome (%) in AML patients

Outcome	Frequency	Percent
Complete Remission	25	45.5%
Resistant	6	10.9%
Incomplete Remission	4	7.3%
Dead	20	36.4%
Total	**55**	**100.0%**

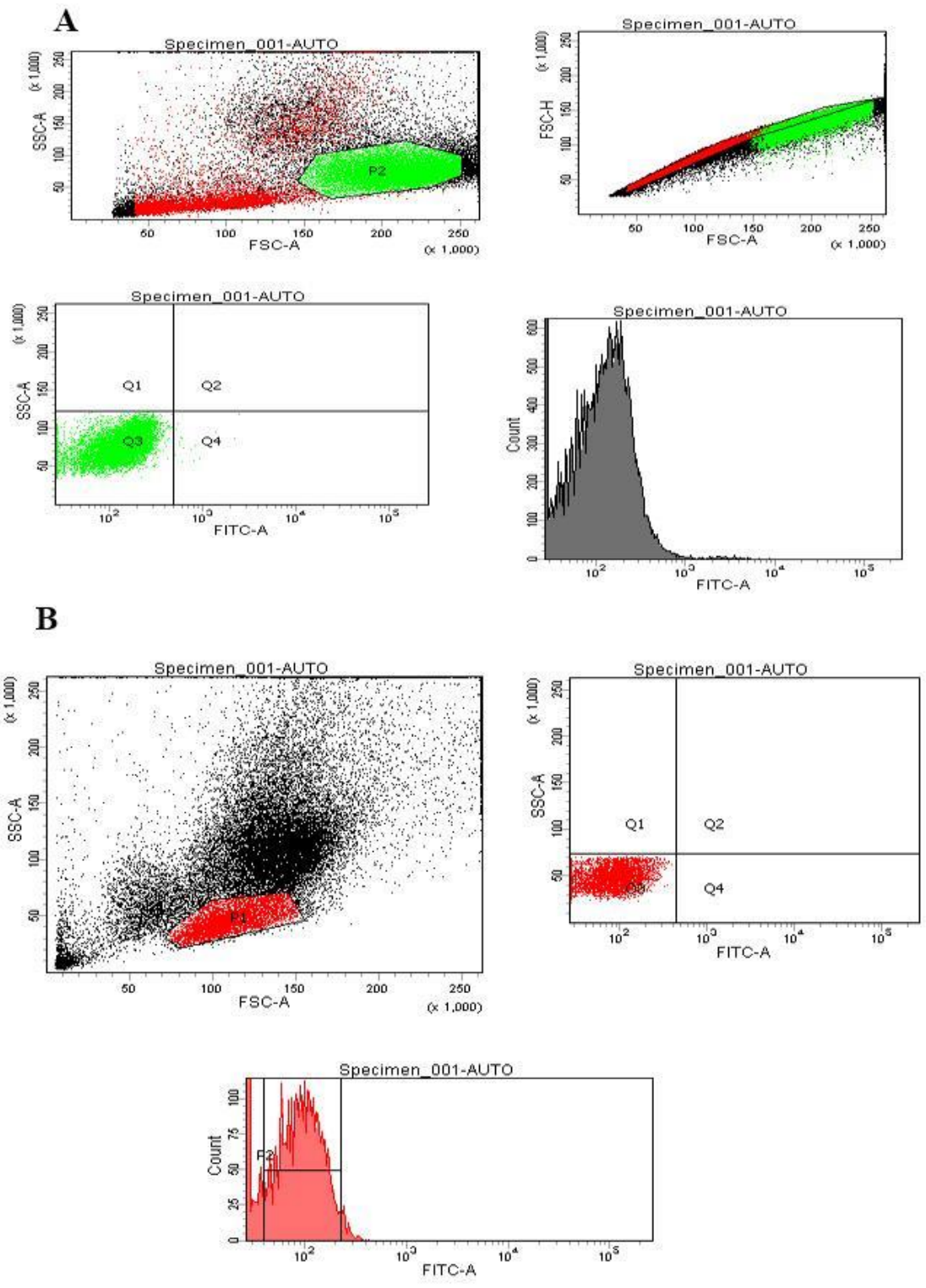
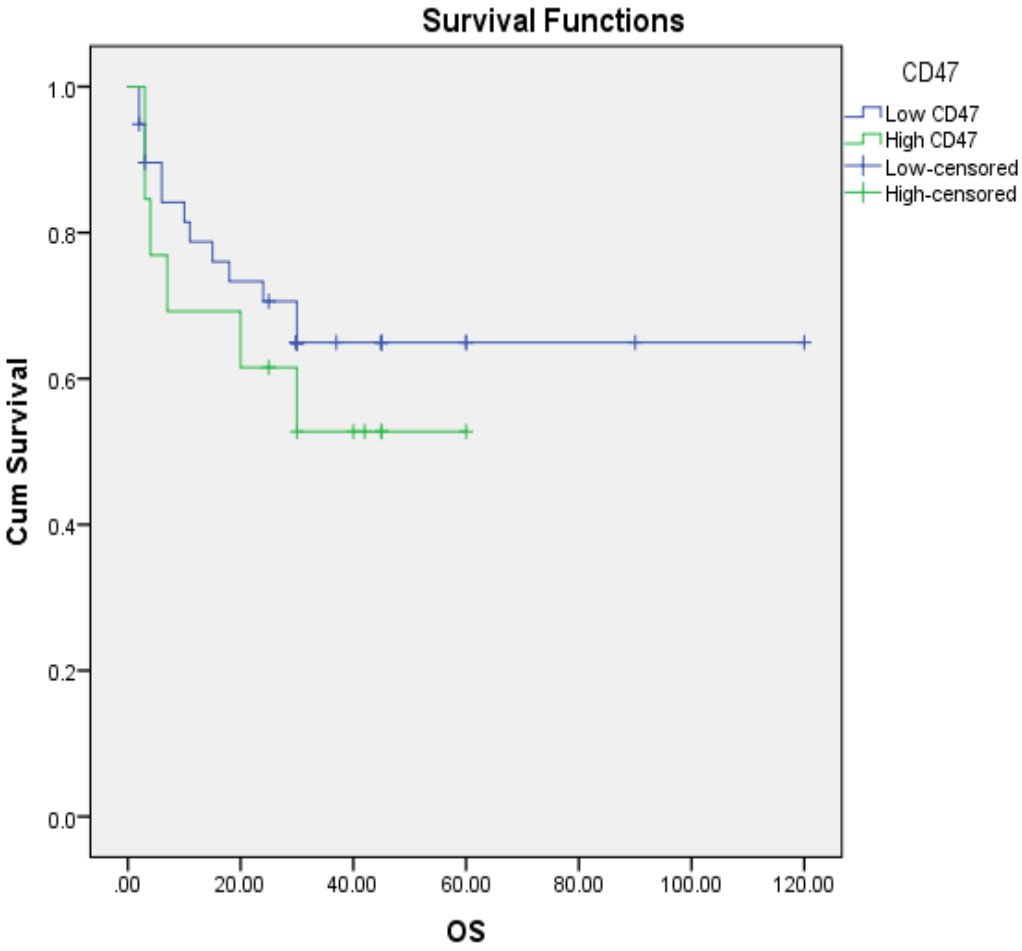


Fig. 1: Flow cytometeric CD47 (as a prognostic marker) analysis showed two samples of AML patients.

3.4 Survival analyses

Survival analyses were carried out using Kaplan Meier curve to examine the expression of CD47 with Over Survival (OS) as shown in **Figure 2.**



Overall Comparisons		
	Chi-Square	P value
Log Rank (Mantel-Cox)	15.9	0.001
Test of equality of survival distributions for the different levels of outcome.		

Fig. 2: Survival analysis of CD47 expression level. The relation between OS (month) and CD47 expression level reported significant as OS reported.

4. DISCUSSION

Acute myeloid leukemia (AML) is a sickness of uncontrolled clonal proliferation of abnormal myeloid stem and progenitor cells in the hematopoietic tissue. The changed myeloid cells or 'leukemic blasts' exhibit aberrant differentiation and accumulate in the bone marrow (BM). This process diminishes ordinary hematopoiesis, regularly leading to thrombocytopenia and anemia, hematopoietic failure and mortality **(Papaemmanuil et al., 2016)**. Structurally, CD47 incorporates an extracellular N-terminal hydrophilic Ig superfamily area and an intracellular hydrophobic domain. Signal regulatory protein alpha (SIRPα) has been recognized as the receptor to CD47 **(Ali et al., 2019)**. Although targeting CD47 represents a unique mechanism of motion and may additionally have broad applicability throughout a number of cancers, the ubiquitous nature of CD47 gives a therapeutic project **(Pietsch et al., 2017)**. One research pronounced robust affiliation between CD47 expression and acute myeloid leukemia, AML is geared up as a cellular hierarchy initiated and maintained via a subset of self-renewing leukemia stem cells **(Majeti et al., 2009)**. The intention of the present day find out about was to look at the CD47 gene expression in Egyptian AML patients for the contrast of its position in the pathogenesis of the sickness and as a prognostic marker associated with usual survival. To achieve our aim, blood samples from 55 patients with AML and 21 healthful individuals as controls have been subjected to a tremendous magnetic selection of CD34+ leukemic cells, and then CD47 expression on these cells used to be estimated through flowcytometry.

CD47 is a detrimental prognostic issue and therapeutic antibody target on human AML stem cells, researchers hypothesized that accelerated CD47 expression on human AML LSC contributes to pathogenesis by using inhibiting their phagocytosis via the interplay of CD47 with an inhibitory receptor on phagocytes **(Majeti et al., 2009)**.

Fallowing medical findings and laboratory investigations had been being carried by all affected persons of AML. These blanketed history/clinical findings, and laboratory investigations. The medical points covered sex, age and the laboratory investigations protected CBC) Specifically Hemoglobin Percentage, Total Leucocyte Count, Platelet Count, and Blast Count: Peripheral Blood Smears, Bone Marrow Aspiration Biopsy Trephine Biopsy, Flow Cytochemistry (Myeloperoxidase staining, Immunohistochemistry (CD47 markers) used to be done.

In the present study, there was a significant difference ($p < 0.0001$) between two studied groups regarding hemoglobin conc., these results was agreed with the result of **(F and Shamsi, 2016)** when found that, hemoglobin levels ranged between 4.3 to 12.5 gm/dl. The majority of patients 49(45.8%) had Hb less than 8.0 gm/dl. About 27% of patients had values between 8 to 10 gm/dl. In the present study, the mean number of WBCs (10^3/µL) in AML patients and healthy were included in the present study. There was a significant difference ($p < 0.0001$) between the two groups. The mean count of PLTs (10^3/µL) in AML patients and healthy were included in the present study. There was a significant difference ($p < 0.0001$) between the two groups. **(F and Shamsi, 2016)** found that, out of 107 patients of AML included in this study 90 patients had leucocytosis accounting to 84.1%, while 7 had Leucopenia, their percentage being 6.5% and only 10 patients had TLC within normal limits. The White cell count was highest in patients with AML-M2 62.5 to 275×10^9/L, followed by AML-M0 with a range of $36\text{-}257\times10^9$/L and, AML-M1 had a range between $36\text{-}257\times10^9$/L while the lowest count

was observed M3, in the range of 18-81 and MDS 2-68×10^9 with an average of 41×10^9. **(F and Shamsi, 2016)** concluded that, overall types of acute myeloid leukemia were more commonly seen in male patients. Age has a significant effect being more common in adults. The frequency of AML increases in adults than children in the 86/14% ratio. The majority of patients were anemic with low hemoglobin levels less than 8 g/dl in 46% cases, leukocytosis and thrombocytopenia.

This results in incidences of CD47 differ significantly among FAP type in AML patients. The mean percent of CD47 were increased with FAP type excretion, 38.6 ± 39.5 at M1, 51.1±34.9 at M2, 60.2±51.9 at M3 while, those showed decrease 33.1±22.6 with M4 and 43.2±31.4 with M5. **(Galli et al., 2015)** reported that, CD47 staining on BM leukemia blasts was scored semi-quantitatively and correlated with scientific parameters and regarded prognostic elements in AML. Low (scores 0-2) and excessive (score 3) CD47 protein expression have been found in 75% and 25% of AML patients. CD47 expression considerably correlated with share BM blast infiltration and peripheral blood blasts. Moreover, excessive CD47 expression was related to nucleophosmin (NPM1) gene mutations.

There was an inverse correlation between concentrations of CD47 gene expression and general survival, with enhanced expression also connected with worse general survival ($P < 0.0001$). This is in accordance with **(Jaiswal et al., 2009)** and **(Majeti et al., 2009)** who conducted their work on leukemic stem cells. Analysis of AML patients disclosed a poorer prognosis associated with greater concentrations of CD47 expression **(Tsai and Dischер, 2008)**. We conclude that in survival analysis for AML patients, CD47 gene expression should be used as a prognostic indicator.

Acknowledgements:

We would like to thank our pricey patients, as properly as all individuals who partly backed this piece of work.

Conflicts of interest:

Authors declared no conflicts of interest

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