

# Low Notch-1 and high Jagged-1 expressions are associated with better treatment response and survival in Adult Egyptian Patients with Acute Myeloid Leukemia

### **Abstract**

**Background:** Acute myeloid leukemias (AMLs) are neoplastic proliferations arising in hematopoietic precursor cells in the bone marrow resulting in overgrowth of myeloblasts and other cells of myeloid lineage. The Notch-1 receptor is a type I transmembrane protein. Interactions between Notch-1 and its ligands Jagged-1 and Dll-1 result in proteolytic cleavages within the receptor, and finally the intracellular domain (Notch-1-IC) is released and translocated in the nucleus. Notch-1 expression was also detected in CD34+ marrow progenitors and other cells in both the bone marrow and the peripheral blood. Jagged-1 and Delta-like1 (Dll-1) seem to be functionally opposite members of the Notch-1 ligand family, but the exact mechanisms of their function in AML remain unclear.

**Methods:** Expression of the Notch-1 intracellular domain and surface expression of Jagged-1 and Dll-1 ligands were assessed in leukemic blasts of newly diagnosed patients with AML by flow cytometry. Additionally, the expression of examined proteins was correlated with clinical data, laboratory data, response to treatment, disease-free (DFS) and overall survival (OS).

**Results:** Notch-1 was positively expressed in 20% of studied patients. Positive Notch-1 expression was associated with shorter OS (6.1 months) and DFS (3.1 months). Higher Notch-1 expression levels were significantly associated with lower remission and higher relapse rates. In contrary, Jagged-1 protein marker was positively expressed in 56% studied patients, those patients showed shorter OS (6.6 months) and longer DFS (3.6 months) compared to negatively expressed ones. Higher Jagged-1 expression levels were significantly associated with higher remission and lower relapse rates. Positive Dll-1

expression was recorded in 30 % patients, yet with no significant relationship with OS and DFS rates as well as clinical outcome after therapy.

**Conclusion:** Both Notch-1 receptor and its ligands, Jagged-1 and Dll-1, seem to be involved in AML pathogenesis, but mainly Jagged-1 and to lesser extent Dll-1 exerts an influence on clinical outcome. High Jagged-1 surface expression is associated with favorable prognosis in patients with AML. However, further studies are necessary to gain a better understanding of Notch-1 and its ligands mechanisms of action in AML.

### **Keywords**

Leukemia; AML; Notch; Flow cytometry.

### **Introduction**

Acute myeloid leukemia (AML) is the most common malignant myeloid disorder in adulthood, characterized by the blockage of myeloid differentiation and blast cell accumulation in the bone marrow (Kumar, 2011).

One of the major challenges in AML, with the exception of promyelocytic acute leukemia, is still related to the high relapse rate after chemotherapy due to the persistence of residual blast cells in the bone marrow (Zhang et al., 2013) (Ito et al., 2013).

Notch is a developmental and evolutionary pathway that controls proliferation, self-renewal, differentiation, and survival of normal and cancer cells. Notch signaling consists of four receptors, Notch1 to Notch4, and five ligands including Jagged1, Jagged2, Dll1, Dll3 and Dll4 (South et al., 2012).

In AML, the role of Notch remains controversial: some authors described Notch expression and activation in AML samples and AML cell lines, but the pathway was slightly activated, as demonstrated by the low expression level of Notch target genes [11-13]. Consistently, they demonstrated that the reactivation of Notch signaling induced apoptosis and differentiation of blast cells into mature cells (Lobry et al., 2013) (Kannan et al., 2013) (Tohda et al., 2005)

By contrast, the activation of Notch pathway in AML has been associated to bad prognosis (Xu et al, 2011). Moreover, patients with hyper-expression of Notch1 displayed poorer overall survival (Sliwa et al, 2014). Recently, Grieselhuber and co-worker identified Notch expression and activation in acute promyelocytic leukemia, demonstrating that both genetic and pharmacologic inhibition of Notch signaling abrogated the enhanced self-renewal of blast cells (Grieselhuber et al, 2013). However, the effect of exogenous micro-environmental Notch signaling on AML cell survival and response to chemotherapy has not yet been evaluated.

Jagged-1 and Delta-like 1 (Dll-1) seem to be functionally opposite members of the Notch-1 ligand family, but the exact mechanisms of their function in AML remain unknown (Chiaramonte et al, 2005) (Tohda et al, 2003).

So, we performed this study to help understanding the role of Notch-1, Jagged-1 and Dll-1 in treatment response and prognosis of AML.

## **Patients and methods**

The present study was conducted on 50 patients with newly diagnosed AML. Cases presented to the Hematology/Oncology unit, Internal Medicine department, Tanta University Hospital and Tanta Oncology Institute during the period from January 2019 to December 2020. The studied patients were 30 males and 20 females with male to female ratio 1.5:1 and their ages ranged from 48 to 61 years.

All patients provided a signed informed consent. Research methods were approved by Tanta Faculty of Medicine Ethics Committee, with approval number 30673/12/15. The study was performed in accordance with the declaration of Helsinki (1964) and revised in 2008.

**Inclusion criteria:** Patients older than 18 years newly diagnosed with AML were included in the study.

**Exclusion criteria:** Patients previously diagnosed with AML and receiving treatment, or patients with any malignant disease other than AML were excluded from the study.

All patients were subjected to the following: full history taking and complete clinical examination, routine laboratory investigations: Complete Blood Count (CBC) including differential count (by ERMA PCE-210N cell counter with examination of peripheral blood films stained with Giemsa stain), LDH, liver enzymes tests were done (by BECKMAN COULTER; AU480; Brea, California, USA), bone marrow aspirates (BMA) were taken and examined for morphology, cytochemical stain and immunophenotyping. Specific laboratory tests include: Flow cytometric analysis of the bone marrow aspirate samples to detect Notch-1, Jagged-1 and Dll-1 expression on leukemic blast cells.

After being fully investigated, all the patients received appropriate treatment in the form of standard induction chemotherapy with daunorubicin and cytarabine (80% of patients). Repeated courses of decitabine or low-dose cytarabine were administered to unfit elderly patients (20% of patients). All trans retinoic acid (ATRA) was added to patients with promyelocytic leukemia (M3).

### **Follow up of the patients**

The patients were observed for 18 months to estimate the overall survival (OS) and disease-free survival (DFS).

OS is measured from the date of diagnosis to the date of death from any cause; patients not known to have died at the last follow up are censored on the date they were last known to be alive.

DFS is measured from the date of end of induction until the date of relapse or death from any cause; patients not known to have relapsed or died at the last follow up are censored on the date they were last examined.

The differences in survival were evaluated using the Kaplan-Meier method

## **Sampling**

Two ml of peripheral venous blood were collected into an EDTA vacutainer tube for complete blood count and Giemsa-stained smears and were labelled. One ml of peripheral blood was delivered into EDTA vacutainer tube and used for immunophenotypic determination. Two ml blood were collected into an empty tube and serum was separated for measurement of serum LDH and liver and renal tests.

## **Flow cytometry of targeted proteins**

### 1. Procedure for intracellular marker (NOTCH-1):

For each sample, two tubes were labeled, one for negative isotopic control and the other for intracellular marker NOTCH-1. The cell count was adjusted to  $10^6$ . The next steps were done to each sample. 100  $\mu$ l of peripheral blood were put in the staining tube. Adding of 2 ml of FACS lysing solution. The tubes were vortexed and incubated in the dark at room temperature for 15 minutes. Spinning down the samples at 1400 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 2 ml of phosphate buffered saline (PBS). Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 400  $\mu$ l of BD permilization solution (BD Cytofix/Cytoperm<sup>TM</sup>) for each sample. Incubation in the dark at room temperature for 15 minutes. Adding of 2 ml of washing buffer (PBS). Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 100  $\mu$ l of PBS. Adding of the aliquots of the antibody, Anti-NOTCH-1 monoclonal antibody PE labeled (BD biosciences), Catalogue number (CN) 560972, clone 9F10, (5  $\mu$ l to the sample tube) and mix well. Incubation in the dark at room temperature for 30 minutes. Adding of 2 ml of PBS. Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 500  $\mu$ l of PBS. Run the samples in the cytometer (Acquisition).

### 2. Procedure for cytoplasmic markers (Jagged-1 and Dll-1):

For each sample, two tubes were labeled, one for negative isotopic control and the other for the monoclonal antibodies. The cell count was adjusted to  $10^6$ . The next steps were done to each sample. 100  $\mu$ l of peripheral blood were put in the

staining tube. Adding the aliquots of the antibodies Anti-JAGGED-1 monoclonal antibody PE labeled (Abcam), Catalogue number (CN) 139943, clone T22-A, anti DLL-1 monoclonal antibody PE labeled (BD biosciences), Catalogue number (CN) 340576, clone 100, and PE labelled mouse isotopic negative control to prevent the nonspecific binding of monoclonal antibodies (background fluorescence intensity). (5 µl of each antibody) to the blood and mix well. Incubation in the dark at room temperature for 30 minutes. Adding of 2 ml of FACS lysing solution. Incubation in the dark at room temperature for 15 minutes. Spinning down the samples at 1400 rpm for 5 minutes. Discard the supernatant and save the pellets. Adding of 2 ml of PBS. Spinning down the samples at 1300 rpm for 5 minutes. Discard the supernatant and save the pellets. Resuspension of the pellets in 500 µl of PBS. Run the samples in the cytometer (Acquisition).

### **Flow cytometric analysis**

FACS caliber flow cytometry from Becton Dickinson was used for analysis. Automated cell quest software was used for data acquisition and analysis. The instrument setting was set by using calibrated beads provided by the manufacturer. Isotopic quality control was used to exclude nonspecific binding and autofluorescence. 10,000 events (cells) at least were passed in front of the laser for each case from which the blast cells were selectively gated (surrounded by a line to separate them from other cells in the basic histogram) for immunophenotyping analysis. Light scatter histogram, forward light scatter versus log side scatter, was used to delineate cell populations of interest (Myeloblasts) by bitmap drawing (gating). Gated fluorescence dot plot is evaluated for positive cells by using cursor position from dot plot for isotopic controls, so that 98% of positives are defined.

### **Interpretation of the results**

After 10000 events were counted, the numbers of blast cells expressing the markers emitting fluorescence signals were summated and multiplied in the PMT2 and the computer analyzed the data as a single-colored frequency histogram.

A case was defined as Notch-1, Jagged-1 or Dll-1 positive if more than or equal to 30% of the gated cells expressed the marker.

## **Statistical analysis**

Statistical presentation and analysis of the study were conducted using the IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, Ill., USA).

Numeric data was presented as mean and standard deviation, while categorical data as number and percentage. The distributions of numeric variables were tested for normality also histogram and QQ plot were used for vision test.

For normally distributed numeric data, Student *t* test was used to compare means of two independent variables. However, Mann Whitney U test was used for non-normally distributed variables. One-way ANOVA test was applied for mean comparison between more than two independent groups of parametric data. However, Kruskal-Wallis test was the alternative for non-parametric data. For categorical data, and chi-square or Fisher's exact tests were used to compare their frequencies.

Correlations between two quantitative variables were assessed using *Pearson* coefficient (*r*). Binary logistic regression, a predictive analysis to examine relationship between numeric independent variables and dichotomous (binary) dependent variable.

## **Survival analysis**

Equality of survival between studied groups was tested by using log rank test, after plotting of data on Kaplan-Meier curve.

Significance test results were quoted as two-tailed probabilities. *P*-value  $\leq .05$  was considered significant.

## **Results**

Clinically, in spite of higher percentage of fever, infection, hepato-splenomegaly, and lymphadenopathy in Notch-1 +ve patients, the difference was not statistically significant. Only TLC was significantly higher in Notch-1 +ve patients when compared to Notch -ve patients. On the other hand, bleeding was significantly more common in Jagged-1 positive patients as shown in (Table 1).

When we studied the different FAB classes in relation to the studied parameters, we could not find a significant association (Table 2).

Interestingly, there was a better treatment response in patients with lower expression of Notch-1, while that was the case with higher expression of Jagged-1. (Table 3).

Binary logistic regression models were performed to predict the potential effect of biomarkers expression on clinical outcome and establish if there's -statistically significant- relationship between these variables. It was noticed that increased notch-1 expression was associated with worse clinical outcome; lower odds of CR (OR = 0.92) and marginally higher odds of relapse (OR = 1.04). However, Jagged-1 was associated with better outcome; higher odds of CR (OR = 1.06) and decreased odds of relapse (OR = 0.96). Nevertheless, Dll-1 expression level proved to non-significant predictor for neither complete remission nor relapse. (Table 4)

On studying OS and DFS, we found that both rates revealed non-significant difference between Notch-1, Jagged-1 and Dll-1 in positive and negative expression groups (p-value= 0.69, 0.54, 0.77 for OS and 0.20, 0.08, 0.64 for DFS) respectively. (**Error! Reference source not found.1**).

Table 1: Clinical and laboratory data of AML patients in relation to Notch-1, Jagged-1 and Dll-1 expression

Parameter	Notch-1			Jagged-1			Dll-1		
	+ve Notch-1 (n=10)	-ve Notch-1 (n=40)	P	+ve Jagged-1 (n=28)	-ve Jagged-1 (n=22)	P	+ve Dll-1 (n=15)	-ve Dll-1 (n=35)	P
<b>Fever / Infection</b>	9 (90%)	29 (72.5%)	0.27	18 (64.3%)	20 (90.9%)	0.2	8 (53.3%)	30 (85.7%)	0.11
<b>Bleeding</b>	8 (80%)	36 (90%)	0.60	27 (96.4%)	17 (77.2%)	0.02*	13 (86.7%)	31 (88.6%)	0.98
<b>HSM</b>	5 (50%)	22 (55%)	0.38	15 (53.5%)	12 (54.5%)	0.39	7 (46.7%)	20 (57.1%)	0.71
<b>LN</b> s	6 (60%)	16 (40%)	0.44	13 (46.5%)	9 (40.9%)	0.3	8 (53.3%)	14 (40%)	0.3
<b>TLC (x10<sup>9</sup>/L)</b>	60.1 ± 24.8	39.5 ± 30.4	0.05*	45.79 ± 33.53	40.9 ± 26.1	0.57	44.1 ± 38.5	43.4 ± 26.7	0.94
<b>Hb (gm/dL)</b>	8.8 ± 1.9	9.6 ± 1.8	0.18	9.19 ± 1.89	9.8 ± 1.7	0.26	9.4 ± 2.0	9.5 ± 1.7	0.88
<b>Platelet count (x10<sup>9</sup>/L)</b>	122.8 ± 71.6	123.8 ± 60.2	0.96	121.89 ± 48.21	125.8 ± 77.0	0.84	125.7 ± 49.8	122.7 ± 67.0	0.88
<b>Peripheral Blasts (%)</b>	27.7 ± 15.6	25.2 ± 23.2	0.75	28.68 ± 21.99	21.9 ± 21.4	0.28	31.9 ± 24.9	23.0 ± 20.1	0.19
<b>BM Blasts (%)</b>	57.3 ± 19.3	51.6 ± 23.9	0.49	57.32 ± 21.92	46.9 ± 23.5	0.11	55.5 ± 25.8	51.5 ± 21.9	0.58
<b>ALT (IU/L)</b>	46.9 ± 36.3	45.1 ± 30.7	0.87	47.32 ± 34.48	43.0 ± 27.9	0.64	43.9 ± 30.5	46.1 ± 32.3	0.82
<b>AST (IU/L)</b>	89.6 ± 67.2	76.3 ± 40.5	0.42	82.39 ± 53.31	74.6 ± 36.9	0.56	78.2 ± 44.4	79.3 ± 48.0	0.94
<b>LDH (U/L)</b>	817.9 ± 432.7	847.8 ± 493.9	0.86	851.28 ± 518.98	829.8 ± 432.5	0.88	992.7 ± 525.8	777.1 ± 448.8	0.17
<b>Uric Acid (mg/dL)</b>	7.3 ± 1.3	7.5 ± 1.6	0.76	7.40 ± 1.61	7.5 ± 1.4	0.92	7.6 ± 1.5	7.4 ± 1.5	0.64

AST: Alanine aminotransferase. **AST**: Aspartate transaminase. **BM**: bone marrow. **Hb**: hemoglobin. **HSM**: hepato-splenomegaly. **LDH**: lactic dehydrogenase. **LN**: lymphadenopathy. **TLC**: total leucocytic count.

Table 2: FAB classification in relation to Notch-1, Jagged-1 and Dll-1 expression

M stage	Notch-1			Jagged-1			Dll-1		
	+ve / -ve	Expression (Mean ± SD)	p	+ve / -ve	Expression (Mean ± SD)	p	+ve / -ve	Expression (Mean ± SD)	p
M0 (n=1)	0 / 1	21.00	0.99	1 / 0	35.00	0.29	0 / 1	8.00	0.65
M1 (n= 2)	0 / 2	21.50 ± 7.78		2 / 0	44.50 ± 4.95		1 / 1	33.00 ± 15.56	
M2 (n=13)	3 / 10	28.46 ± 23.47		4 / 9	25.08 ± 22.59		5 / 8	26.77 ± 18.48	
M3 (n= 5)	1 / 4	20.80 ± 5.63		4 / 1	56.80 ± 24.71		3 / 2	33.40 ± 21.82	
M4 (n= 8)	1 / 7	26.25 ± 23.38		6 / 2	42.75 ± 20.82		1 / 7	21.75 ± 14.76	
M5 (n=16)	4 / 12	27.13 ± 25.49		9 / 7	41.25 ± 27.28		3 / 13	21.00 ± 12.55	
M6 (n=5)	1 / 4	24.20 ± 26.40		2 / 3	30.60 ± 28.80		2 / 3	27.00 ± 21.00	

Table 3: AML outcome in relation to Notch-1, Jagged-1 and Dll-1 expression

Treatment Response		Notch-1				Jagged-1				Dll-1			
		+ve	-ve	Expression	p	+ve	-ve	Expression	p	+ve	-ve	Expression	p
Complete Remission	Yes (n=21)	2 (9.5%)	19 (90.5%)	15.3 ± 8.0	0.001*	13 (61.9%)	8 (38.1%)	53.4 ± 25.5	0.001*	8 (38.1%)	13 (61.9%)	26.8 ± 16.6	0.44
	No (n= 29)	8 (27.6%)	21 (72.4%)	33.8 ± 25.4		15 (51.7%)	14 (48.3%)	26.5 ± 17.8		7 (24.1%)	22 (75.9%)	23.1 ± 16.3	
Relapse	Yes (n=15)	5 (3.3%)	10 (66.7%)	38.3 ± 27.8	0.04*	6 (40%)	9 (60%)	24.5 ± 17.5	0.04*	3 (20%)	12 (80%)	22.3 ± 17.0	0.51
	No (n=35)	5 (14.3%)	30 (85.7%)	20.8 ± 16.7		22 (62.9%)	13 (37.1%)	43.5 ± 25.9		12 (34.3%)	23 (65.7%)	25.7 ± 16.1	
Death	Yes (n=14)	3 (21.4%)	11 (78.6%)	29.1 ± 22.5	0.55	9 (64.3%)	5 (35.7%)	28.5 ± 18.5	0.06	4 (28.6%)	10 (71.4%)	24.0 ± 16.1	0.86
	No (n=36)	7 (19.4%)	29 (80.5%)	24.9 ± 21.9		19 (52.8%)	17 (47.2%)	41.4 ± 26.5		11 (30.6%)	25 (69.4%)	24.9 ± 16.6	

Table 4: Logistic regression of the studied biomarkers expression level in complete remission and relapse:

Response	Biomarkers expression level%	Odds Ratio	95% CI	p-value
CR	Notch-1	0.92	0.86 - 0.99	0.03*
	Jagged-1	1.06	1.02 - 1.09	0.001*
	Dll-1	1.01	0.98 - 1.05	0.43
Relapse	Notch-1	1.04	1.01 - 1.07	0.02*
	Jagged-1	0.96	0.94 - 0.99	0.02*
	Dll-1	0.99	0.95 - 1.03	0.50

CI: confidence interval. CR: complete remission. P: p value.

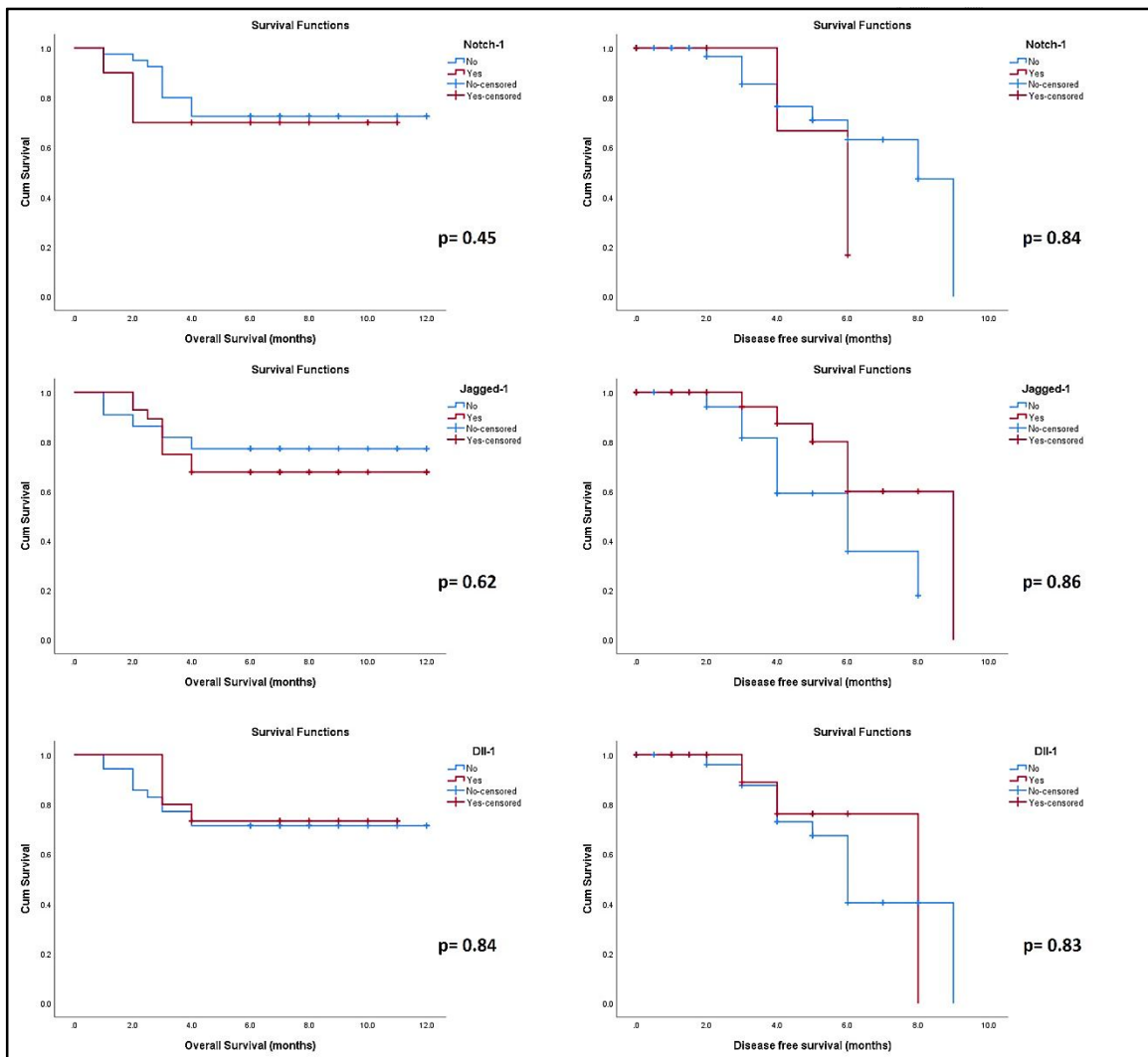


Figure 1: Kaplan-Meier curves for overall survival and disease-free survival in relation to Notch-1, Jagged-1 and Dll-1 expression

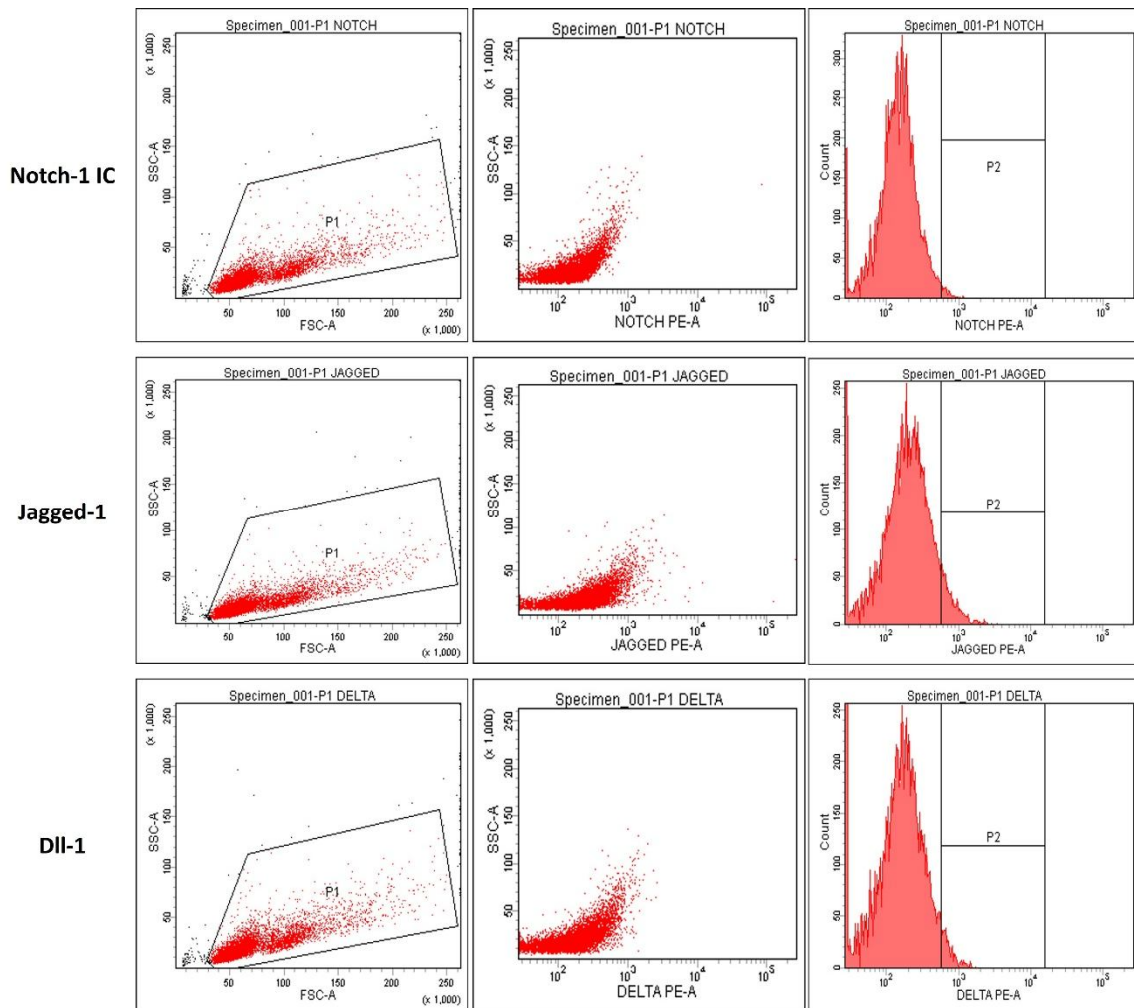


Figure 2: Expression patterns of Notch-1-IC, Jagged-1 and Dll-1 in AML blasts

## Discussion

AML is a major challenge for personalized medicine. Thanks to the association of advanced diagnostic procedures, including genome-wide molecular-profiling, immunophenotyping, cytogenetic, and clinical features, many biomarkers guiding therapeutic strategy have been defined (Prada-Arismendy et al, 2017)

Notch receptors are single-pass transmembrane proteins which play a critical role in cell-fate decisions and have been implicated in the regulation of many developmental processes. Notch receptors transduce short-range signals by interacting with the

transmembrane Delta-like and Jagged ligands on neighboring cells. (Gragnani et al, 2021)

Importantly, the role of aberrant Notch signaling in hematological malignancies, has also emerged as an exciting field of cancer research. Oncogenic Notch1 receptor mutations are present in over 50% of T-cell acute lymphoblastic leukemias (T-ALL) (Weng et al, 2004). Interestingly, in the case of acute myeloid leukemia (AML) the role of the Notch pathway is not well understood, with different reports describe contrasting results.

In AML the role of Notch remains controversial (Takam et al, 2021). Kannan et al described weak activation of the Notch pathway, as demonstrated by the low expression level of the Notch target genes. (Kannan et al, 2013) Similarly, Lobry et al. (2013) described epigenetic silencing of Notch target genes in AML; consistently, they demonstrated that the reactivation of Notch signaling induced apoptosis and differentiation of leukemia blast cells into mature cells. These results are consistent with the anti-leukemic role of demethylating/hypomethylating agents azacytidine or decitabine in AML (DiNardo et al., 2018; Leung et al., 2019). However, other groups found that Notch activation is not homogenous within AML samples and cell lines (Sliwa et al., 2014; Czemerska et al., 2014).

Hereby, in this study we aimed to evaluate the clinical role of Notch-1, Jagged-1 and Dll-1 in 50 adult patients newly diagnosed as AML. We have searched for the potential relationship between expression of these biomarkers in one hand and clinical presentation, certain laboratory findings, and clinical outcomes as well as survival rates after therapy. The expression pattern of Notch-1-IC, Jagged-1 and Dll-1 was represented by the multicolored fluid cytometry in AML cases.

Notch-1 was positively expressed in only 20% of the cases at mean expression level of  $64.10 \pm 19.65$  % and negatively expressed in the remaining 80% with mean expression level of  $16.55 \pm 7.01$  %. While Jagged-1 and Dll-1 markers were positively expressed in 56% and 30% and negatively expressed in 44% and 70% of cases, respectively. Their mean positive expression levels were  $56.14 \pm 17.07$  % and  $47.13 \pm 7.03$  %, respectively.

Different expression pattern was recorded by Czemerska et al., who analyzed the expression examined proteins (Notch-1, Jagged-1, Dll-1) in leukemic cells and peripheral blood stem cells as a control. They found no significant difference in Notch-1-IC expression in both groups and significantly higher expression levels of Jagged-1 and Dll-1 in AML blasts than in control group. (Czemerska et al, 2014) However, they performed their study on bone marrow leukemic blasts not peripheral blood blast as we did.

On breakdown of relationship between expression levels and FAB classes, we found non-significant difference in protein expression of the 3 markers relative to different FAB classes.

In their flow cytometric study, Takam et al examined protein expression in blast samples collected from 79 newly diagnosed AML patients. They reported much higher Notch-1 and Jagged-1 expression rates (85.51% and 86.08%, respectively) and much lower Dll-1 expression rate (5.80%). (Takam et al, 2019). This difference in expression level could be explained by higher presentation of M0-M1 FAB subtypes (17.22%) relative to this study (6%). It is believed that these less mature subtypes are associated with protein overexpression as documented by Sliwa et al., who found significant correlation between hyperexpression of Notch-1 and the morphological subgroups M0-M1. (Silwa et al, 2014)

In this study we evaluated the relationship between protein markers expression and different laboratory data, such as Hemoglobin level, total leukocytic count, platelet count, peripheral and bone marrow blast counts, serum enzyme (ALT, AST and LDH) and serum uric acid level. Of these parameters, only significant relationship was found between positive Notch-1 expression and significantly higher leukocytic count ( $60.10 \pm 24.83 \times 10^9/L$ ), however all other relationships were proven to be insignificant.

Czemerska et al reported non-significant correlations between Notch-1, Jagged-1 and Dll-1 proteins expression levels and the same laboratory data. (Czemerska et al, 2014) On the other hand, Takam et al. reported negative association of TLC with Notch-1 expression as well as positive associations between platelet count with Notch-1 and Hb level with Jagged-1. (Takam et al, 2019)

In this study, Notch-1 expression was linked with poor clinical outcome. Only 20 % of Notch-1 positive cases (2 patients) had complete remission. However, complete remission was linked to significantly lower mean Notch-1 level ( $15.33 \pm 7.99$  %) compared to non-remission cases ( $33.83 \pm 25.38$  %). Additionally, relapsed cases expressed significantly higher levels of Notch-1 compared to non-relapsed ones ( $38.27 \pm 27.81$  % vs.  $20.83 \pm 16.68$  %). Although there was better OS and DFS in negative than in positive Notch-1 expression, yet the difference was not reaching statistical significance.

Our results were in agreement with those of Czemreska et al., who described that high Notch-1 expression was associated with lower but non-significant CR rates (76%) compared to low Notch-1 expression (58%). (Czemerska et al, 2014).

Various other reports have provided similar evidence that high levels of Notch-1 are associated with poor prognosis of AML cases. Aref et al. studied Notch-1 gene mutations in bone marrow samples obtained from 50 AML patients. They reported that unmutated cases had significantly higher CR rate (77%) and longer OS (21.2 months) compared to the mutated ones (0% and 1.2 months, respectively). (Aref et al, 2020)

Moreover, Sliwa et al., in their retrospective study used immunohistochemical analysis of 97 AML bone marrow biopsies. They found that cases with Notch-1 protein overexpression in blast cells had a significant inferior OS and 1-year survival (14%) rates compared to cases without Notch-1 hyperexpression (48%) (Sliwa et al, 2014). Xu et al. also assessed the prognostic value of Notch-1 expression in bone marrow mononuclear cells using real-time PCR. They reported significantly shorter relapse-free and overall rates ( $8.3 \pm 1.9$  and  $22.8 \pm 2.6$  months, respectively) in the patients with higher Notch-1 expression relative to those with low Notch-1 expression ( $13.8 \pm 2.5$  and  $38.7 \pm 3.3$  months). (Xu et al, 2011)

In contrast with Notch-1, positive Jagged-1 expression in AML patients was found to be related to good prognosis. Complete remission was associated with significantly higher Jagged-1 expression ( $53.43 \pm 25.53$  %) in comparison with cases that did not show CR ( $26.45 \pm 17.79$ %). Meanwhile, positive Jagged-1 expressors (n: 28) experienced relapse in 6 patients only (21.43%) and the remaining 22 (78.57%) had no relapse. Moreover, the

relapsed cases had significantly lower expression levels of Jagged-1 compared to non-relapsed cases ( $24.53 \pm 17.50\%$  vs.  $43.46 \pm 25.85\%$ , respectively). However, there was no significant difference in OS and DFS between positive and negative Jagged-1 expressors.

Similar findings were reported by Czemreska et al. who demonstrated that CR rate of the high Jagged-1 expressors was higher than low expressors (70% vs. 52%). But unlike us, they found a significant association between positive Jagged-1 expression and increased overall survival rate (Czemerska et al, 2014). Nevertheless, different results were obtained Xu et al. who reported that higher Jagged-1 gene expression was associated with significantly shorter DFS ( $9.8 \pm 1.3$  months vs.  $13.2 \pm 1.7$  months) and OS rates ( $24.6 \pm 3.5$  months vs.  $38.5 \pm 2.8$  months) compared to cases with lower Jagged-1 expression (48%) (Xu et al, 2011). This discrepancy could be explained by the different methodology adopted in Xu et al. study (real-time PCR for unselected bone marrow mononuclear cells) making a room for sample contamination by non-leukemic BM cells and thus influencing PCR results.

According to our study, we failed to detect significant association between clinical outcome (CR or relapse) and Dll-1 expression levels despite the strong tendency of Dll-1 positive cases to avoid relapse (only 3 of 12 Dll-1 positive cases had relapse attack).

Other studies showed some controversial results regarding Dll-1 expression. Czemreska et al. reported non-significant lower CR rate with high Dll-1 expression (53% vs. 63%) with no relationship found between survival and Dll-1 expression (Czemerska et al, 2014). Conversely, Xu et al. found significant decrease in DFS and OS rates with high rather than low Dll-1 expression ( $9.9 \pm 1.0$  months vs.  $13.6 \pm 2.1$  months and  $24.1 \pm 3.6$  months vs.  $38.6 \pm 3.2$  months, respectively) (Xu et al, 2011). It is to be noted that Xu and colleagues detected high Dll-1 expression in 54% of cases compared to 30% positive Dll-1 cases in our study.

Our results suggest an important role of Notch-1 and Jagged-1 in the treatment response and outcome. Which might serve as a potential therapeutic target. Gu et al reported that AML cell growth arrest and caspase-dependent apoptosis could be induced through

activation of each of the four Notch receptors. suggesting the potential therapeutic use of Notch agonists in the treatment of AML (Gu et al, 2016). While Takam et al reported that pan-inhibition of Notch using the Notch transcription factor inhibitor SAHM1, reduced AML cell proliferation without any effect on cell death (Takam et al, 2016)

## **Conclusion**

Negative Notch-1 and positive Jagged-1 are associated with better treatment response and outcome in AML patients, which favors using them as potential prognostic biomarkers in AML. Furthermore, they may be targeted for treatment of AML.

## **References:**

- Aref S, Rizk R, El Agdar M, et al. NOTCH-1 gene mutations influence survival in acute myeloid leukemia patients. *Asian Pacific J Cancer Prev.* 2020;21(7):1987-1992.
- Chiaramonte R , Basile A , Tassi E , et al . A wide role for NOTCH1 signaling in acute leukemia . *Cancer Lett* 2005; 219: 113– 120.
- Czemerska, M., Pluta, A., Szmigielska-Kaplon, A., Wawrzyniak, E., Cebula-Obrzut, B., Medra, A., et al. (2014). Jagged-1: a new promising factor associated with favorable prognosis in patients with acute myeloid leukemia. *Leuk. Lymphoma* 56, 401–406.
- DiNardo, C. D., Pratz, K. W., Letai, A., Jonas, B. A., Wei, A. H., Thirman, M., et al. (2018). Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol.* 19, 216–228.
- Gragnani L, Lorini S, Marri S and Zignego A. Role of Notch Receptors in Hematologic Malignancies. *Cells* 2021, 10, 16.
- Grieselhuber NR, Klco JM, Verdoni AM, Lamprecht T, Sarkaria SM, Wartman LD and Ley TJ. Notch signaling in acute promyelocytic leukemia. *Leukemia.* 2013; 27:1548- 1557.
- Gu Y, Masiero M, and Banham A. Notch signaling: its roles and therapeutic potential in hematological malignancies. *Oncotarget,* 2016; 7(20): 29804-29823.

- Ito S, Barrett AJ, Dutra A, Pak E, Miner S, Keyvanfar K, Hensel NF, Rezvani K, Muranski P, Liu P, Melenhorst JJ and Larochelle A. Long term maintenance of myeloid leukemic stem cells cultured with unrelated human mesenchymal stromal cells. *Stem Cell Res.* 2015; 14:95- 104.
- Kannan S, Sutphin RM, Hall MG, Golfman LS, Fang W, Nolo RM, Akers LJ, Hammitt RA, McMurray JS, Kornblau SM, Melnick AM, Figueroa ME and Zweidler-McKay PA. Notch activation inhibits AML growth and survival: a potential therapeutic approach. *J Exp Med.* 2013; 210:321- 337.
- Kumar CC. Genetic abnormalities and challenges in the treatment of acute myeloid leukemia. *Genes Cancer.* 2011; 2(2):95-107.
- Leung, K. K., Nguyen, A., Shi, T., Tang, L., Ni, X., Escoubet, L., et al. (2019). Multiomics of azacitidine-treated AML cells reveals variable and convergent targets that remodel the cell-surface proteome. *Proc. Natl. Acad. Sci. U.S.A.* 116, 695–700.
- Lobry C, Ntziachristos P, Ndiaye-Lobry D, Oh P, Cimmino L, Zhu N, Araldi E, Hu W, Freund J, Abdel-Wahab O, Ibrahim S, Skokos D, Armstrong SA, Levine RL, Park CY and Aifantis I. Notch pathway activation targets AML-initiating cell homeostasis and differentiation. *J Exp Med.* 2013; 210:301-319.
- Prada-Arismendy J, Arroyave JC, and Röthlisberger S. Molecular biomarkers in acute myeloid leukemia. *Blood Rev.* 2017;31(1):63-76.
- Sliwa T, Awsa S, Vesely M, Rokitte D, Grossschmidt P, Jilch R, Ulrich W and Geissler K. Hyperexpression of NOTCH-1 is found in immature acute myeloid leukemia. *Int J Clin Exp Pathol.* 2014; 7:882-889.
- South AP, Cho RJ and Aster JC. The double-edged sword of Notch signaling in cancer. *Semin Cell Dev Biol.* 2012; 23:458-464.
- Takam Kanga P, Bassi G, Cassaro A, Midolo M, Di Trapani M, Gatti A, Carusone R, Resci F, Omar Perbellini, Michele Gottardi, Massimiliano Bonifacio, Armel Hervé Nwabo Kamdje, Achille Ambrosetti and Mauro Krampera. Notch signaling drives bone marrow stromal cell-mediated chemo-resistance in acute myeloid leukemia. *Oncotarget*, 2016; 7(16): 21713-21727.

- Takam Kamga, P., Collo, G. D., Resci, F., Bazzoni, R., Mercuri, A., Quaglia, F. M., et al. (2019). Notch signaling molecules as prognostic biomarkers for acute myeloid leukemia. *Cancers* 11:1958.
- Takam Kamga P, Bazzoni R, Dal Collo G, Cassaro A, Tanasi I, Russignan A, Tecchio C and Krampera M (2021) The Role of Notch and Wnt Signaling in MSC Communication in Normal and Leukemic Bone Marrow Niche. *Front. Cell Dev. Biol.* 8:599276
- Tohda S , Murata-Ohsawa M , Sakano S , et al . Notch ligands, Delta-1 and Delta-4 suppress the self-renewal capacity and longterm growth of two myeloblastic leukemia cell lines . *Int J Oncol* 2003 ; 22 : 1073 – 1079 .
- Tohda S, Kogoshi H, Murakami N, Sakano S and Nara N. Diverse effects of the Notch ligands Jagged1 and Delta1 on the growth and differentiation of primary acute myeloblastic leukemia cells. *Exp Hematol.* 2005; 33:558-563.
- Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, and Aster JC. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004; 306: 269-71.
- Xu X, Zhao Y, Xu M, Dai Q, Meng W, Yang J and Qin R. Activation of Notch signal pathway is associated with a poorer prognosis in acute myeloid leukemia. *Med Oncol.* 2011; 28 Suppl 1:S483-489.
- Zhang B, Li M, McDonald T, Holyoake TL, Moon RT, Campana D, Shultz L and Bhatia R. Microenvironmental protection of CML stem and progenitor cells from tyrosine kinase inhibitors through N-cadherin and Wnt-beta-catenin signaling. *Blood.* 2013; 121:1824-1838.