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Prognostic Significance of Brain and Acute Leukemia, Cytoplasmic (*BAALC*) Gene Expression in Adult Egyptian Patients with Acute Myeloid Leukemia with Normal Cytogenetics

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ABSTRACT

Background: Acute myeloid leukemia with normal cytogenetics (CN-AML) comprises 45% of adult de novo AML and detection of predictive molecular marker might improve response to therapy.

Aim: Study of impact of brain and acute leukemia, cytoplasmic (*BAALC*) gene expression on prognosis of acute myeloid leukemia with normal cytogenetics.

Patients and Methods: *BAALC* expression was determined by real-time reverse transcriptase polymerase chain reaction (RT-PCR) in bone marrow samples of 48 adult de novo (CN-AML). Patients were classified at *BAALC*'s median expression into high and low expressers. *BAALC* expression was analyzed in context of other clinical and hematological parameters

Results: Twenty four (50%) patients showed high *BAALC* expression (above median level) and 24 (50%) showed low *BAALC* expression (below median level). Sixteen (76.2%) of the patients with low *BAALC* expression responded to induction chemotherapy compared to 9 (42.8%) with high *BAALC* expression (p=0.03). There was no association between *BAALC* expression and any of the hematological or clinical parameters except for association between high *BAALC* and CD34 expression (p=0.001).

Progression free and overall survival was not significantly different between high and low *BAALC* expressers but there was a trend of worse survival in high expressers.

Conclusion: High *BAALC* expressers have a bad prognosis and molecular assessment of *BAALC* at diagnosis might be of prognostic value in CN-AML patients.

Key Words: *AML – CN-AML – BAALC gene.*

INTRODUCTION

Acute myeloid leukemia (AML) is a life-threatening hematopoietic stem cell neoplasm, it is cytogenetically and molecularly heterogeneous disease characterized by clonal proliferation of myeloid precursors and maturation arrest with accumulation of acquired genetic alterations in hematopoietic progenitor cells that disturb normal mechanisms of cell growth, proliferation and differentiation **[1]**.

The abnormal expression of genes and epigenetics is closely connected with the occurrence and development of AML and it plays an important role in the pathogenesis and prognosis of AML. Among factors associated with adverse prognosis in AML, the overexpression of the human gene brain and acute leukemia, cytoplasmic (*BAALC*) **[2]**.

BAALC is a gene located on chromosome 8q22.3. DNA sequence and expression pattern were highly conserved among mammals. It encodes a protein of yet unknown function **[3]**.

Where as normal blood and bone marrow show very low levels of *BAALC* expression, high levels of *BAALC* transcript can be detected in hematopoietic progenitor cells as well as in leukemic blasts in some AML patients. *BAALC* expression in normal bone marrow is restricted to the compartment of progenitor cells and shows high expression in a subset of leukemic blasts. Hence, *BAALC* may be seen as a stage-specific marker that maintains proliferative capacity and inhibits differentiation in a regulated way during hematopoiesis but if aberrantly expressed, this can lead to leukemogenesis **[4]**.

BAALC expression is considered an independent prognostic factor in cytogenetically normal acute myeloid leukemia (CN-AML). Also as high *BAALC* expression predicts an adverse prognosis, it may be defined as an important risk factor in AML with normal cytogenetics **[5,6]**.

In this study, we analyzed the expression of the *BAALC* gene using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in 48 pretreatment bone marrow samples of CN-AML patients to evaluate its prognostic value.

PATIENTS AND METHODS

This study was carried out on 48 consecutive newly diagnosed CN-AML patients who presented to the Medical Oncology Department, National Cancer Institute (NCI), Cairo University. Patients included 21 males and 27 females with an age range of 18-67 median 38 years. Patients were followed-up for 1-18, median 11 month.

Diagnosis was established after clinical, morphological, cytochemical, flow cytometric and cytogenetic analysis. All cases met the AML diagnostic standards.

The study was approved by NCI Institutional Review Board (IRB) and a written informed consent was obtained from all study subjects.

Clinical end points:

Complete remission (CR) is defined as bone marrow blasts <5%; absence of blasts with Auer rods; absence of extra-medullary disease; absolute neutrophil count >1.0 x 10^9/L; platelet count >100 x 10^9/L; independence of red cell transfusions. Treatment failure includes either resistant disease or relapse. Resistant disease is defined as failure to achieve CR following completion of initial treatment with evidence of persistent leukemia by blood and/or bone marrow examination. Relapse is defined as bone marrow blasts ≥5 percent, reappearance of blasts in the blood or development of extra-medullary disease.

Overall survival (OS) is defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Progression free survival (PFS) is defined as the time from starting therapy until documented progression or death. For patients without disease progression (DP) at the time of analysis, the date of last follow-up was considered right-censored **[7]**.

Sample collection, RNA extraction and cDNA synthesis:

Bone marrow samples (1ml) were collected on EDTA from patients with AML. Bone marrow was treated with erythrocyte lysis solution; Leukocytes were collected and stored in buffer RLT ($1x10^7$ leukocytes) at –80°C till use for RNA extraction. Total RNA was extracted using QIAamp RNA extraction blood Mini kit (QIAGEN) following the standard procedures according to the manufacturer's instructions. The amount of RNA was measured by nanodrop spectrophotometer at 260 and 280 wave length; a ratio of 1.8-2:1 denoted good quality of RNA. Subsequently,1.0µg RNA was reverse transcribed into cDNA in 20µL reaction using random hexamer according to manufacturer's instructions (High capacity cDNA reverse transcription kit) (Applied Biosystems, USA) and stored at –20°C till use.

Molecular detection of BAALC gene:

Relative gene expression analysis was performed on an ABI PRISM 7500 Detector system (Applied Biosystems, Foster City, CA) by two step quantitative RT-PCR assays using Quanti Fast Probe Assay Duplex Detection (Qiagen, Hilden, Germany). Amplification was performed in a 25µl volume including 250ng of cDNA as template. Each sample was assayed in duplicate and the analysis was duplicated using cDNA from two independent RT reactions. Universal Taqman Master Mix including High-Rox dye solution was used.

Amplification conditions were: 5 minutes at 95°C as PCR initial activation step and HotStarTaq Plus DNA Polymerase activation, followed by 40 cycles at 95°C for 30 seconds (denaturation), and 60°C for 30 seconds (combined annealing/extension).

Primer probes (Qiagen, Hilden, Germany), optimized for use with Quanti Fast Probe Assay Duplex Detection Kit, were Mm-*BAALC*-1 (QF00108178) (Fam dye) for *BAALC* and Hs-

GABDH-2 (QF00470722) for glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) (VIC dye). Data were reported as –ΔΔCT using *GAPDH* as a reference gene. The median value of gene expression was taken as a cut off to divide the patients into high and low *BAALC* expressers.

Statistical methods:

Data were analyzed using SPSS version 20. Categorical data were summarized as percentages. Numerical data were summarized as mean ± standard deviation or median and range if skewed.

OS and PFS were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha of 0.05 level, with a 95% confidence interval.

RESULTS

BAALC gene expression had a range of 0.000394–0.158220 in the patients group with a median of 0.010710. Twenty four patients had values higher than the median (high expressers) and 24 had lower values (low expressers).

Demographic and laboratory characteristics of patients are summarized in Table (1).

No statistically significant differences were encountered between both groups with regards to age, gender, organomegaly or lymphadenopathy (Table 2).

Neither were there significant differences between high and low *BAALC* expressers with regards to Hb level, TLC, platelet count or percentage of BM blasts at first presentation or FAB classification (Table 3).

Patients with high *BAALC* expression showed a tendency towards higher blast percentage in peripheral blood at presentation than those with low *BAALC* expression (p=0.09).

Patients were assessed for response on day 28, out of the 48 studied patients, 42 were evaluable for response. Complete remission (CR) was achieved in 25 patients (59.452%), 5 (11.9%) failed to achieve CR, and 12 (28.57%) died during induction.

Complete remission was achieved in 16/21 low (76.19%) as compared to 9/21 (42.86%) high *BAALC* expressers (p=0.03, Table 4).

At the end of follow-up period: 18 patient (85.71%) in the high and 14 (66.67%) in the low *BAALC* expressers died. Median OS was 2ms (95% CI 0.71-3.28) and 5ms (95% CI 3.54-6.46) respectively. (p=0.21, Fig. 1).

In high *BAALC* expressers 6 out of 9 patients relapsed (66.67%), with a median PFS of 6ms (95% CI 3.381-8.619). In the low *BAALC* expressers 10/16 patients relapsed (62.5%) with a median PFS of 4ms (95% CI 1.060-6.94) (p=0.845, Fig. 2).

Table (1): Clinical and laboratory characteristics of 48 adult acute myeloid leukemia patients.

Parameter	Finding	Parameter	Finding
Age:			
Years	38 (18-67)*	BM Cellularity	
Gender:			
Male	21.0 (43.75)**	Hyper-cellular	44 (91.3)**
Female	27.0 (56.25)**	Normo-cellular	4 (8.7)**
Organomegaly	18 (37.5)**	*CD34:*	
		Positive	25.0 (54.3)**
		Negative	21.0 (45.7)**
Lymphadenopathy	7.0 (14.58)**	FAB	
TLC x10^9/L	37.0 (1.7-207)*	M0	2 (4.2)**
Hb: gm/dl	7.2 (3.0-11.4)*	M1	11 (22.9)**
Platelet x/10^9L	29.0 (5.0-458)*	M2	14 (29.2)**
PB blasts	60.5 (3-98)*	M4	19 (39.2)**
BM blasts	54.5 (12-90)*	M5	2 (4.2)**

*Median (range). **No (%).
TLC: Total leukocytic count. Hb : Hemoglobin.
PB : Peripheral Blood. BM: Bone marrow

Table (2): Demographic and clinical features of 48 acute myeloid leukemia patients in relation to *BAALC* gene expression.

Parameter	*BAALC* expression		p-value
	High No: 24	Low No: 24	
Age:			
Years	39.5±11.7*	37.7±16.1	0.14
Gender:			
Male	8 (38%) **	13 (62%)	0.15
Female	16 (59.3%)	11 (40.7%)	
Organomegaly	7 (38.9%)	11 (61.1%)	0.26
Lymphadenopathy	5 (71.4%)	2 (28.6%)	0.41

*Mean ± S.D. **No (%).

Table (3): Hematological parameters in 48 acute myeloid leukemia patients in relation to *BAALC* gene expression.

Parameter	*BAALC* expression		*p*-value
	High No: 24	Low No: 24	
Hemoglobin: gm/dL*	6.6±2.2	7.3±1.9	0.279
Total leukocytic count: x 10^9/L**	105.3 (3.6-207)	71.85 (1.7-142)	0.752
Platelets: x 10^9/L**	231.5 (5-458)	153.5 (6-301)	0.423
Peripheral blood blast %**	50 (3-97)	60.6 (23-98)	0.093
Bone marrow blast %**	55 (20-90)	51 (12-90)	0.687
*FAB classification***:*			
M0, M1	9 (69.2%)	4 (30.8%)	
M2	6 (42.9%)	8 (57.1%)	0.267
M4, M5	M4, M5	12 (57.1%)	
*CD34 expression***:*			
Positive	19 (76%)	6 (24%)	<0.001*
Negative	5 (21.71%)	18 (78.26%)	

*Mean ± S.D. **Median (range). ***No (%).

Table (4): Response to induction chemotherapy in 48 adult acute myeloid leukemia patients in relation to *BAALC* gene expression.

Treatment outcome	*BAALC* expression		*p*-value
	High No: 21	Low No: 21	
Complete remission	9 (42.86%)*	16 (76.19%)	
Resistant	3 (14.2%)	2 (9.52%)	0.03
Death during induction	9 (42.86 %)	3 (14.29%)	

*No (%).

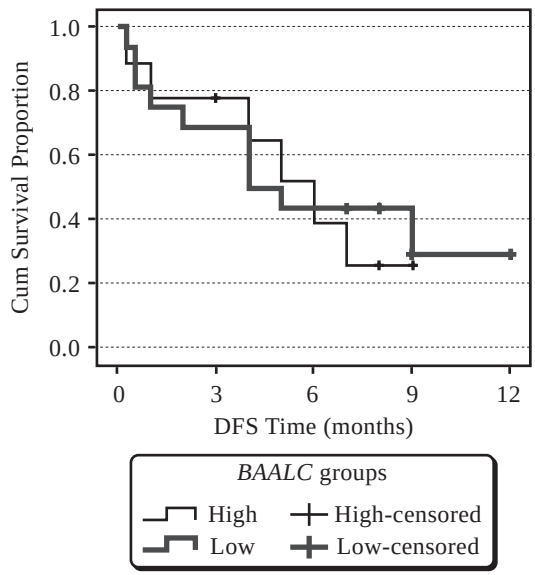

Fig. (1): Disease free survival for patient with high and low *BAALC* expression p = (0.85).

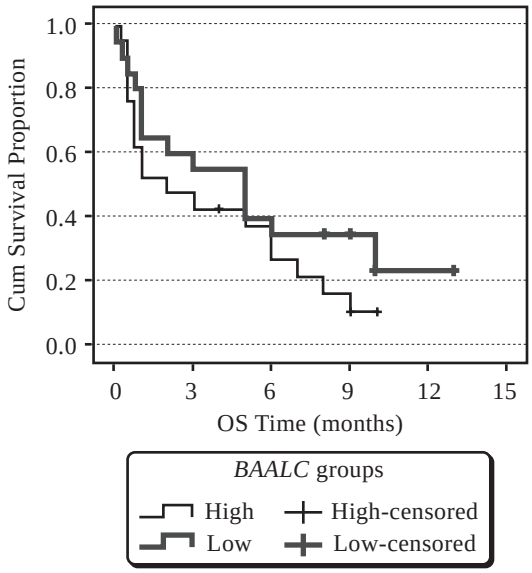

Fig. (2): Overall survival for patient with high and low *BAALC* expression. p = (0.21).

DISCUSSION

In this work we evaluated the *BAALC* gene expression in 48 CN-AML cases. Patients were judged as high or low expressers according to the median level of *BAALC* expression. Previous studies evaluated *BAALC* expression by real-time PCR considering the median level obtained in each study as the discriminator between high and low expression **[3,4]**.

In the present work, no statistically significant differences were encountered between high and low *BAALC* gene expresser CN-AML patients with regards to age, gender, organomegaly or lymphadenopathy. This is in agreement with two previous studies **[4,8]**. One previous study reported male predominance **[9]** and another reported female predominance **[10]**.

In the current study, no statistically significant differences were encountered between high and low *BAALC* gene expresser CN-AML patients with regards to Hb level, TLC, platelet count or number of blasts in bone marrow at presentation. This is in agreement with previous studies **[8,4,11,12]**. However, we encountered a trend for higher PB blast% at presentation in the high expresser group (p=0.09). This is supported by the findings of Baldus et al. **[8]** who reported 56% PB blasts at presentation in high vs. 31% in low *BAALC* expressers (p=0.004). Similar findings were detected in a previous

Egyptian study **[12]**. Failure of achieving statistical significance in our study may be attributed to our small sample size. The association of high *BAALC* expression with higher PB blasts may be explained by the effect of *BAALC* gene on hematopoietic progenitors; it inhibits differentiation of the progenitors and favors their proliferation **[3]**.

Previous studies reported high *BAALC* expression in more immature FAB subtypes M0, M1 and low *BAALC* in M5 subtype **[8]**. However no statistically significant association was detected with FAB subtypes in our study; this may be attributed to the small number of patients.

In the current study *BAALC* over expression was associated with CD34 expression (*p*-value <0.001), a finding that is consistent with previous studies **[11,12]**; they reported that high *BAALC* levels were frequently detected in CD34 positive cases. Their finding and ours is in harmony with the assumed role of *BAALC* gene in inhibiting differentiation and favoring proliferation of hematopoietic progenitors **[4]**.

In the current study, failure of remission induction either due to death in induction or failure to reduce the blast count to less than 5% of nucleated marrow cells, was significantly higher in high than in low *BAALC* gene expressers. This is in agreement with previous studies that reported a higher rate of primary resistance and refractoriness to chemotherapy among high *BAALC* gene expressers **[8,11,12,13]**.

Previous studies reported high *BAALC* expression as an independent prognostic factor for inferior PFS and OS **[5,8]**. In our study no statistically significant difference was found in OS or PFS between high and low *BAALC* expressers although a trend towards higher OS was noticed in low *BAALC* expressers. This can be explained by the small number of patients in our study.

In conclusion, we found that high *BAALC* expression is associated with unfavorable course of disease; patients are more resistant to treatment. Thus molecular assessment of *BAALC* at diagnosis may be of prognostic value in AML. A higher number of cases and longer follow-up and further studies are needed to validate this assumption.

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