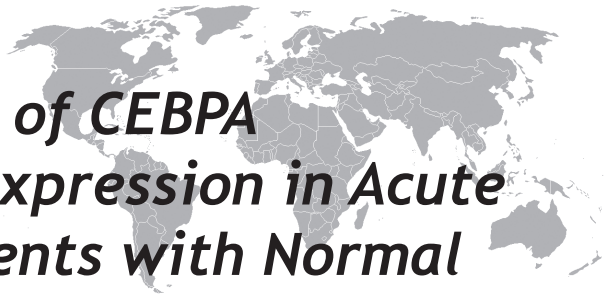

Prognostic Significance of CEBPA Mutations and BAALC Expression in Acute Myeloid Leukemia Patients with Normal Karyotype

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ABSTRACT

Aim: *The aim of this work is to study the prognostic impact of mutations in the myeloid transcription factor gene CEBPA (for CCAAT/enhancer binding protein-a) and expression of the BAALC gene (for brain and acute leukemia, cytoplasmic), a novel gene involved in leukemia, in 38 adults with AML and normal cytogenetics.*

Method: *Screening for mutations of CEBPA gene was assessed using PCR-single-strand conformation polymorphism (PCR-SSCP), and BAALC expression was determined by real-time reverse transcriptase polymerase chain reaction in blood or bone marrow samples.*

Result: *CEBPA mutations were found in 7 (18.4%) of 38 patients, 36.8 % (14 of 38) had low BAALC expression and 63.2 % (24 of 38) had high BAALC expression. Patients with CEBPA mutations had favorable course of their disease. They had higher rate of complete remission (CR) (85.7 % vs 51.6 %; p= 0.108), lower incidence of relapse (0% vs 41.9%; p= 0.038). Disease free survival (DFS) and overall survival (OS) were significantly longer for patients with CEBPA mutations compared with patients without mutations (mean 13.65±5.41 vs 7.32±4.33 months, p= 0.047; mean 15.32±6.5 vs 8.5±3.21 months, p= 0.039; respectively). Compared to low BAALC expressers, high BAALC expressers had lower incidence of CR (50% vs 71.4%; p= 0.171), higher incidence of relapse (50% vs 14.3%; p= 0.029), and showed significantly shorter DFS (mean 7.5±2.12 vs 11.67±4.6 months, p= 0.038) and inferior overall survival (mean 9.1±3.52 vs 13.22±4.21 months, p= 0.024).*

Conclusion: *From this study, we can conclude that CEBPA mutation status and BAALC expression are important prognostic factors in AML patients with normal cytogenetics and their incorporation into novel risk-adapted therapeutic strategies will improve the currently disappointing cure rate of this group of patients.*

Key words: *Prognostic marker, CEBPA, BAALC , leukemia*

INTRODUCTION

Acute myeloid leukemia (AML) is clinically and molecularly heterogeneous disease. Currently, cytogenetic findings provide the most important prognostic information and are used to guide risk-adapted treatment strategies (1,2). However, by conventional cytogenetic techniques, karyotype abnormalities are detected in only half of all AML cases and the other half are commonly described as normal-karyotype AML (3,4) and therefore lack informative chromosome markers (2,5). Patients with normal-karyotype AML usually have an intermediate risk with a 5- year overall survival of between 35% and 45% (3,6), but they are very heterogeneous with regard to their pretreatment features and response to therapy (7), as well as acquired gene mutations and changes in gene expression in bone marrow or blood (8). Thus, the identification of molecular markers that precisely differentiate a patient's risk could improve treatment outcome by the use of sophisticated risk adaptive treatment strategies (9,10).

CCAAT/ enhancer binding protein alpha (C/EBPα) is a transcription factor involved in the regulation of myelopoiesis (11,12). It is encoded by CEBPα gene that is located at chromosome 19q13.1 (13). Like other members of the basic region leucine zipper (bZIP) class of transcription factors, it possesses a bipartite DNA-binding domain composed of a positively charged basic (b) region that contacts the DNA and a leucine zipper (ZIP) in the C terminus that mediates dimerization. The less-conserved N terminus contains regulatory and transcription domains (14). In hematopoiesis, CEBPA plays a piovotal role in early stages of myeloid differentiation. It is particularly expressed in myelomonocytic cells (15) and is specifically up- regulated during granulocytic differentiation (5). On the basis of the observation that C/EBPα deficient mice lack mature granulocytes, it has been speculated that CEBPα mutations might contribute to the differentiation block specific to AML (16).

Mutations of C/EBPα gene affect either the NH2-terminal part of the protein that prevent expression of the full-length (42 kDa) protein and result in truncated non functional isoform (30 kDa) with dominant negative activity (17). The remaining mutations are identified at the C-terminus of C/EBPα and predict mutant proteins lacking DNA binding and/ or homodimerization activity. Some patients present with biallelic mutations at the C-terminus, whereas others are heterozygous for separate mutations or found to have a C-terminal mutation coexisting with a mutation in the N-terminus (18). Interestingly, the presence of a CEBPα mutation was associated with significantly better clinical outcome (16).

BAALC (Brain And Acute Leukemia, Cytoplasmic) is a recently identified gene on chromosome 8q22.3 and implicated in normal hematopoiesis. It encodes a protein with no homology to any known proteins or functional domains (19). Expression of BAALC is found mainly in neuroectoderm-derived tissues and hematopoietic precursor cells. In hematopoietic cells, BAALC expression is restricted to the compartment of progenitor cells whereas no expression was detected in mature bone marrow or circulating white blood cells (20). In leukemias, high BAALC expression is found in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML) in blast crisis, whereas no BAALC expression can be detected in patients with chronic-phase CML or chronic lymphocytic leukemia (CLL). High BAAL expression levels were first identified in a study of AML patients with trisomy 8 as a sole abnormality (19). Trisomy 8 has been associated with poor prognosis in AML (1,2), and it is therefore hypothesized that BAALC expression may assist in prognosis of AML patients lacking cytogenetic aberrations.

The aim of the present study is to assess the presence of mutations in the CEBPA gene and of the expression of the BAALC gene in AML patients with normal cytogenetics. In addition, these markers were analyzed for correlation with clinicohematologic features and outcome.

MATERIAL AND METHODS

Subjects

Thirty eight patients with AML were diagnosed, treated, and had been followed up until death or for periods up to 24 months (between 2006 and 2008) in the Internal Medicine Department, Tanta University Hospital. Their ages ranged from 17 to 54 years (mean 35.7±8.25). In addition, 12 healthy volunteers with matched age and sex were included in the study. Cases were selected for analysis on the basis of sample availability [peripheral blood (PB), bone marrow (BM) and the presence of cytogenetic and clinical information].All samples of either PB or BM were enriched by Ficoll-hypaque (1.0779/mL, Amersham Pharmacia, Buckinghamshire, U. K.) density-gradient centrifugation and cryopreserved in 10% fetal bovine serum at -70˚ C until use. Morphologic subtypes of AML were classified according to the French-American-

British (FAB) criteria (21). As a result, 8 patients had M1, 14 had M2, 5 had M4, 10 had M5 and one patient had M6. All patients were managed by combination chemotherapy consisting of double induction and consolidation chemotherapy. Double induction therapy consisted of cytarabine 200 mg/m^2/d continuous infusion for 7 days (day 1-7); and daunorubicin 50 mg/m^2/d for 3 days (days1-3); and etoboside 100 mg/m^2/d for 3 days (ADE), followed by a second course of ADE started between days 21 and 28 in patients responded to the first course. Patients not achieving complete remission (CR) received a course of cytarabine 3000 mg/m^2 every 12 hours on days 1 through 3; mitoxantrone 12 mg/ m^2 on days 2 and 3 (HAM). Consolidation therapy consisted of two cycles of HAM (22). Patients younger than 55 years of age were referred in their first CR to receive allogeneic stem-cell transplantation if an HLA-compatible sibling donor was available (23).

Cytogenetic analysis

Pretreatment cytogenetic analyses of bone marrow (BM) or peripheral blood (PB) were performed. Metaphase chromosomes were banded by G-banding technique and Karyotyped according to the International System for Human Cytogenetic Nomenclature. A minimum of 20 metaphases was required to be examined for a patient to be classified as having normal cytogenetics (2).

Mutational and Expression Analysis

Total cellular RNA was extracted from Ficoll density gradient centrifugation enriched mononuclear cells using QIAmp RNA Blood Mini Kit (Qiagen, Chatsmorth, CA), and complementary DNA (cDNA) was synthesized from 2 µg total RNA applying the Superscript system and random hexamer primers (Invitrogen, San Diego, CA).

CEBPA mutational analysis by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP)

Genomic DNA was isolated from mononuclear cell preparations stored at -70˚ C using QIAamp DNA blood Mini Kit (Qiagen GmbH, Germany). Four overlapping primer pairs were used to amplify the entire CEBPA coding region (GenBank accession number U34070). The sequence of primers were:

1F 5'-GGCGAGCAGGGTCTCCGGGT-3'

1R 5'-TGTGCTGGAACAGGTCGGCCA-3'

2F 5'-GCTGGGCGGCATCTGCGA-3'

2R 5'-CCCCGACGCGCTCGTACAGG-3'

3F 5'-CCGGCTACCTGGACCTGGACGGCAGG-3'

3R 5'-CGTTGCTGTTCTTGTCCACCGACTTCTT-3'

4F 5'-CTCGGTGCCGCCGGCCT-3'

4R 5'-AACCACTCCCTGGGTCCCCGC-3'.

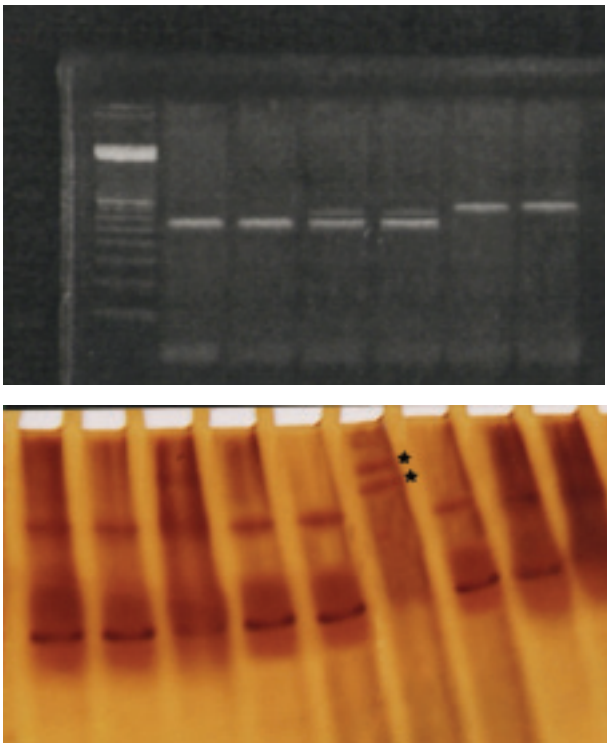
The total reaction volume of 50 µL contained 500 ng DNA, 10 pmol of each primer, deoxynucleotide triphosphates (10 mmol/L each), 2.5 U Taq polymerase, a polymerase chain reaction (PCR) additive facilitating amplification of GC-rich templates, and supplied buffer (Qiagen, Hilden, Germany). Samples were amplified using the following PCR conditions: 95˚C for 15 minutes; 35 cycles of 95˚C for 1 minute, 68˚C for 3 minutes; and 68˚C for 3 minutes (16). PCR products were mixed with 10 volumes of loading buffer and denatured at 96˚C for 5 minutes, quenched on ice immediately, and applied to 10% polyacrylamide gel electrophoresis at 50 V, overnight. Then, stained by silver nitrates, wrapped in plastic foil. Normal CEBPA exhibits a specific conformational pattern. A mutant gene displays pattern with different electrophoretic mobility (mobility shift) (24) (Figure 1).

BAALC mRNA expression by quantitative realtime PCR

BAALC mRNA expression was normalized to the simultaneously analyzed glucose phosphate isomerase (GPI) gene. The relative BAALC expression was determined using the comparative cycle threshold (CT) method. Glucose phosphate isomerase (GPI) and BAALC were coamplified in the same tube using 1 µL cDNA, 1× master mix (IQ Mix; BioRad, Munich, Germany), GPI probe (5'-HEX-TTCAGCTTGACCCTCAACACCAAC-TAMRA-3') with GPI forward (5'-TCTTCGATGCCAACAAGGAC-3') and reverse (5'-GCATCACGTCCTCCGTCAC-3') primers, and BAALC probe (5'-FAM-CTCTTTTAGCCTCTGTGGTCTGAAGGCCAT-TAMRA-3') with BAALC forward (5'-GCCCTCTGACCCAGAAACAG-3') and reverse (5'-CTTTTGCAGGCATTCTCTTAGCA-3') primers. Reactions were performed using real-time PCR 7000 sequence detection system (Applied Biosystems, Foster City, USA). The comparative cycle threshold (CT) method was used to determine the relative expression levels of BAALC, and the cycle number difference (Δ CT = CT GPI – CT BAALC) was calculated for each sample. Relative BAALC expression values are expressed as 2 (ΔCT) (21) (Figure 2).

Statistical Analysis

Baseline clinical features across groups were compared using x2 or a two-sided Fisher's exact test for categoric and the nonparametric Mann-Whitney U test and t-test for continuous variables. A P value less than 0.05 is considered significant. Complete remission (CR) required an absolute neutrophil count of at least 1500/µL, a platelet



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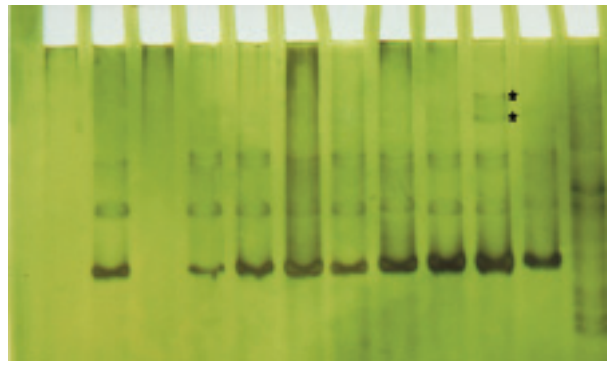

C

B

Figure 1. *CEBPa mutation detection. (A) PCR amplification of CEBPa. Line 1 is molecular weight marker, lane 2 and 3 are wild types, lane 4 and 5 are heterozygous with presence of one allele in addition to wild type allele. Lane 6 and 7 are homozygous with no evidence of the wild-type allele. (B) SSCP of PCR products showing abnormally migrating bands in lane 10 (mobility shift) indicated by asterisk (*) in addition to bands with normal mobility (i.e. heterozygous). The other lanes are derived from genomic DNA samples of other AML patients and display bands with normal mobility. (C) SSCP of PCR products illustrating abnormally migrating bands in lane 6 (mobility shift) indicated by asterisk (*) without the presence of normally migrating bands (i.e. homozygous). The other lanes are derived from genomic DNA samples of other AML patients and display bands with normal mobility.*

count of at least 100× 109/L, no peripheral blood blasts, bone marrow cellularity more than 20% with maturation of all cell lines, no Auer rods, less than 5% bone marrow blasts, and no extramedullary leukemia (25). Relapse was defined as the reappearance of circulating blasts, or greater than 5% blasts in the marrow not attributable to another cause, or development of extramedullary leukemia. Disease-free survival (DFS) was defined only for those patients achieving a CR. It was measured from the CR date until date of relapse or death, regardless of cause, censoring for patients alive at last follow-up. Overall survival (OS) was measured from the protocol on-study date until the date of death regardless of cause, censoring for patients alive at last follow-up. DFS and OS were analyzed using the Kaplan-Meier method, and the log-rank test was used to compare differences between survival curves. All data analyses were done using SPSS software, version 12.

RESULTS

CEBPA mutations and clinical features

In the present study, mutations of the CEBPA gene were found in 7 of 38 AML patients with a normal karyotype (18.4%). Patients with CEBPA mutations differed in many aspects from patients with a wild-type CEBPA gene status. As indicated in Table 1, patients with CEBPA mutations had significantly higher hemoglobin levels (p= 0.012), lower platelets counts (p= 0.031), and were less likely to present with lymphadenopathy or extramedullary leukemia. Their leukocyte count (WBC) tended to be lower and PB blast counts higher, but the difference was not statistically significant (p= 0.096, p= 0.148; respectively). In addition, the median lactate dehydrogenase (LDH) value at diagnosis was not elevated in patients with CEBPA mutations compared with patients without CEBPA mutations (p= 0.012). On the other hand, there were no significant differences in age, sex ratio, or bone marrow blasts between CEBPA-mutated and CEBPA-nonmutated patients. Also, CEBPA mutations were limited to the myeloplastic subtypes of AML (M1 and M2) but were absent in the monocytic (M4 and M5) and erythroblastic (M6) subtypes.

The clinical course was different in AML patients with or without CEBPA mutations. A complete remission after induction chemotherapy was achieved in 6 of 7 (85.7%) AML patients with CEBPA mutations and only in 16 of 31 (51.6%) patients without mutations, but the difference was not statistically significant (p= 0.108). On the other hand, none of the patients with CEBPA mutations relapsed, but relapse occurred in 13 of 31 (41.9%) patients without CEBPA mutations (p= 0.038). Figure 3a and b illustrate that patients without CEBPA mutations had a

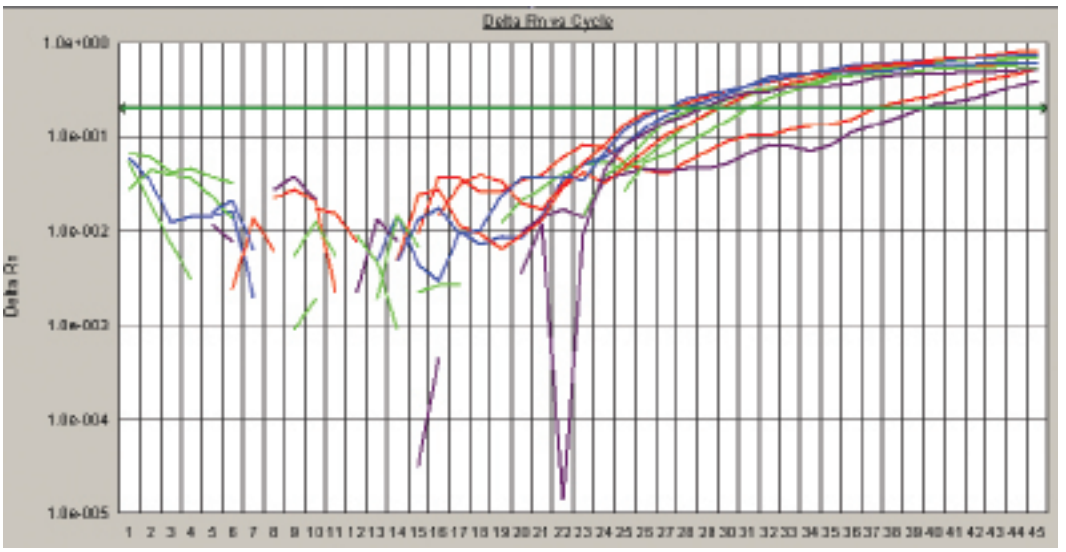

***Figure 2**. Positive expression of BAALC (6 cases) and positive GPI expression by quantitative real-time PCR.*

significantly shorter DFS (mean 7.32±4.33 months) and OS (mean 8.5±3.21 months) than patients with CEBPA mutations (mean 13.65±5.41 months and 15.32±6.5 months, p= 0.047, P= 0.039; respectively) as summarized in Table 2. Thus, we can say that AML patients with a normal karyotype and CEBPA mutations have a favorable course of their disease.

BAALC expression and clinical features

The range of BAALC expression among the 12 volunteers was remarkably small (range, 0.02 and 0.13; median, 0.08). We used the median value of 0.08 of these 12 volunteers as cutoff. Therefore, a value above 0.08 was considered "high expression", whereas a value below 0.08 qualified for "low expression". BAALC expression levels in 38 normal-karyotype AML ranged from 0.003 to 38.5. In the current study, low BAALC mRNA expression levels were found in 14 of 38 AML patients (36.8%), whereas 24 of 38 AML patients (63.2%) demonstrated high BAALC expression levels. The clinicohematologic features differed from patients with low BAALC and those with high BAALC mRNA expression levels. Patients with low BAALC expression had a significantly higher hemoglobin level (p= 0.011) and lower platelet counts (p= 0.047). On the other hand, there were no significant differences between high BAALC and low BAALC-expressing patients with respect to age, sex, leukocytic count (p= 0.087), peripheral blood blast counts (p= 0.136), bone marrow blasts (p= 0.864), LDH value (p= 0.160), or other presenting physical findings such as lymphadenopathy or extramedullary leukemia (Table 3). The clinical course was also different in AML patients as regard BAALC expression. Ten (71.4%) of low BAALC and 12 (50%) of high BAALC expressers achieved CR (p= 0.171). Again, AML patients with high BAALC expression tended to relapse more frequently than those with low BAALC expression (50% versus 14.3%, p= 0.029) (Table 4). High BAALC expression predicted significantly shorter DFS (mean 7.5± 2.12 versus 11.67± 4.6 months, p= 0.038, Figure 3c). Overall survival was also significantly shorter in patients with high BAALC expression compared to those with low BAALC expression (mean 9.1±3.52 versus 13.22±4.21 months, p= 0.024, Figure 3d). Thus, we can predict that AML patients with a normal karyotype and low BAALC expression may have good prognosis.

Ultimately, a multivariable analysis was conducted to determine if CEBPA mutations and BAALC expression were significant independent prognostic markers for DFS and OS in normal-karyotype AML once the model was adjusted for other characteristics. Variables considered for model inclusion were age, WBCs, hemoglobin, platelets, LDH value, percentage of blasts in bone marrow and blood, CEBPA mutation status and BAALC expression. After adjusting for other covariables, CEBPA mutations and BAALC expression remained significant predictors for DFS and OS (Table 5). Patients with CEBPA-wild type were 0.125 (95% CI: 0.024- 0.321) times more likely to die than patients with CEBPA mutations. Also, CEBPA mutation status was a

Table 1. Patient characteristics according to CEBPA mutation status

Variables		CEBPA mutation (n:7)	Wild type (n:31)	p-value
Age, years	Range	(23-49)	(17-54)	0.583
	Mean	36.52±12.75	39.68±13.82	
Sex, n		2F/5M	16F/15M	0.249
Hemoglobin level, g/dL	Range	(6.2-12.5)	(5.1-10.7)	0.012*
	Mean	9.55±1.62	8.08±1.23	
Platelet count, $\times 10^9$/L	Range	(10-190)	(20-310)	0.031*
	Median	84	105	
WBCs, $\times 10^9$/L	Range	(10-45)	(15-73)	0.096
	Mean	28.32±9.52	33.75±8.91	
% blood blasts	Range	(5-72)	(24-66)	0.148
	Mean	47.36±18.17	38.35±13.75	
% bone marrow blasts	Range	(36-90)	(32-98)	0.735
	Mean	63.75±22.73	67.85±29.81	
*LDH, U/L	Range	(102-2183)	(165-3094)	0.012*
	Median	426	1060	
FAB classification n (%)	M1	2(28.57%)	6(19.35%)	0.060
	M2	5(71.43%)	9(29.03%)	
	M4	0(0.00%)	5(16.13%)	
	M5	0(0.00%)	10(32.26%)	
	M6	0(0.00%)	1(3.23%)	
Lymphadenopathy, n (%)		1(14.3%)	8(25.8%)	0.461
Extramedullary involvement, n (%)		0(0%)	4(12.9%)	0.426

significant predictor for DFS. The hazard ratio for CEBPA-wild type patients to fail treatment after achieving CR, in the form of relapse or death was 0.066 (95% CI: 0.02-0.25). Patients with high BAALC expression were 4.215 (95% CI: 2.312- 6.345) times more likely to die than patients with low BAALC expression and the hazard ratio for DFS in patients with high BAALC expression was 3.98 (95% CI: 1.92- 5.123). From this result, it seems that CEBPA mutations and BAALC expression can be strong independent predictors for outcome in normal-karyotype AML.

DISCUSSION

The prognostic effect of various chromosomal aberrations in AML is well established with implications for therapy (4). This study evaluated the prognostic impact of CEBPA gene mutations and BAALC mRNA expression in 38 AML patients with normal cytogenetics. CEBPA mutations were detected in 18.4% of patients. Others have reported slightly lower percentages with between 4.3% and 15% (14,26-28). However, most of these studies have not focused on normal-karyotype AML. Regarding FAB classi-

Table 2. *Impact of CEBPA mutation status on clinical outcome*

Variables		*CEBPA*		*P-value*
		mutation (n=7)	*Wild type (n=31)*	
Complete remission (CR) (%)		*6 (85.7%)*	*16 (51.6%)*	*0.108*
Relapse (%)		*0 (0 %)*	*13 (41.9%)*	*0.038**
DFS, months	*Range*	*(2-22)*	*(0-12)*	*0.047**
	Mean±SD	*13.65±5.41*	*7.32±4.33*	
OS, months	*Range*	*(4-26)*	*(0-14)*	*0.039**
	Mean±SD	*15.32±6.5*	*8.5±3.21*	

NOTE*: DFS and OS are given as a median and also at the time point 2 years after diagnosis.*

fication, this study, in accordance with others, confirmed that CEBPA mutations preferably occur in the FAB classes M1 and M2 (14,16,27-29).

The clinical effect of CEBPA mutations in this study seems to be distinctly favorable. At diagnosis, patients with CEBPA mutations had significantly higher hemoglobin levels, lower platelet counts, lower WBCs counts, lower LDH levels, less lymphadenopathy and extramedullary involvement. The frequency of complete remission (CR) was higher and relapse rate was significantly lower in patients with CEBPA mutations than in patients with wild type. Also, disease free survival (DFS) and overall survival (OS) were significantly longer for patients with CEBPA mutations. These results were confirmed in multivariable analyses, in which CEBPA mutation status was identified as an independent prognostic marker affecting DFS and OS (Hazard ratio= 0.066, p= 0.001, Hazard ratio= 0.125, p= 0.002; respectively). Similar results were also obtained in other studies (4,8,16,18) where CEBPA mutations were prognostic for DFS and OS. These observations suggest that CEBPA mutations may define a distinct biologic subclass of AML with normal cytogenetics (16).

Against our results are the results of Snaddon et al. (29) who found no significant differences in demographic variables, response to therapy, remission duration, and median survival between those intermediate risk patients possessing CEBPA mutations and those without mutations.

It has been shown that patients with CEBPA mutations had similar prognosis as patients belonging to the 'favorable' group according to MRC classification (including t(8;21), inv(16) and t(15;17) AML). It is difficult to speculate on the reasons of this favorable prognosis of AML with CEBPA mutations. However, there are some similarities between the cellular effects of the chimeric oncoproteins observed in AML with 'favorable' karyotype and the cellular effects of CEBPA mutations found in AML with normal karyotype (belonging to the 'intermediate' karyotype subgroup of MRC classification). In both situations, CEBPA function is repressed. Indeed. Chimeric proteins induce the disruption CEBPA normal functions: in t(8;21), AML1-ETO is able to suppress CEBPA protein expression by decreasing the level of CEBPA mRNA; in inv(16), CBFb-MYH11 is also able to disturb CEBPA functions (30) and in t(15;17), Truong et al. (31) showed that PML-RARa blocked CEBPA activity. Those similarities could contribute to explain why AML patients with CEBPA mutations have favorable prognosis (15).

We propose to assess BAALC expression to obtain further prognostic information in normal-karyotype AML patients. High BAALC expression was detected in 63.2% of patients and is predominantly seen in the FAB subtypes M1, M2, whereas high expression levels were less frequent in the monocytic FAB subtypes M4, M5. This result is in agreement with the results of Baldus et al. (32) and Bienz et al. (4). At diagnosis, patients with low BAALC expression had significantly higher hemoglobin levels, lower platelet counts, lower WBCs counts, lower peripheral blood blasts and less lymphadenopathy and extramedullary involvement. As regard the clinical outcome, we found that the frequency of CR was higher and the relapse rate was significantly lower in patients with low BAALC expression than in patients with high expression. Also, patients with low BAALC expression had a significantly better clinical

Table 3. *Patient characteristics according to BAALC expression status*

Variables		BAALC		p-value
		Low BAALC (n:14)	High BAALC (n:24)	
Age, years	Range	(19-49)	(17-54)	0.415
	Mean	35.21±13.51	38.75±12.31	
Sex, n		8F/6M	10F/14M	0.279
Hemoglobin level, g/dL	Range	(5.1-12.5)	(5.5-11.8)	0.011*
	Mean	9.60±0.95	8.7±1.03	
Platelet count, $\times 10^9$/L	Range	(10-280)	(22-310)	0.047*
	Median	50	58	
WBCs, $\times 10^9$/L	Range	(11-52)	(10-73)	0.087
	Mean	32.82±12.75	40.97±14.35	
% blood blasts	Range	(12-72)	(5-60)	0.136
	Mean	47.73±18.75	36.35±12.62	
% bone marrow blasts	Range	(32-96)	(40-98)	0.864
	Mean	65.32±26.53	66.75±23.71	
*LDH, Units/L	Range	(190-3094)	(102-2060)	0.160
	Median	1092	980	
FAB classification n (%)	M1	1(7.14%)	7(29.17%)	0.001*
	M2	1(7.14%)	13(54.17%)	
	M4	4(28.57%)	1(4.17%)	
	M5	8(57.14%)	2(8.33%)	
	M6	0(0.00%)	1(4.17%)	
Lymphadenopathy, n (%)		1(7.1%)	4(16.7%)	0.381
Extramedullary involvement, n (%)		2(14.3%)	4(16.7%)	0.612

outcome than high expressors both for DFS and OS. The significance of BAALC expression as an independent prognostic factor was confirmed by multivariable analysis. In multivariable analysis, high BAALC was independently predictive of inferior DFS and OS (Hazard ratio= 3.98, p= 0.003, Hazard ratio= 4.215, p= 0.001; respectively). Our results are in good agreement with the previous results, showing inferior DFS and OS for high BAALC patients (4,20,30). Also, Bienz et al. (4) concluded that BAALC expression appears to be particularly useful as a prognostic

Table 4. *Impact of BAALC expression on clinical outcome*

Variables		*BAALC*		*p-value*
		Low (n:14)	*High (n:24)*	
Complete remission (CR) (%)		*10 (71.4%)*	*12 (50%)*	*0.171*
Relapse (%)		*2 (14.3%)*	*12 (50%)*	*0.029**
DFS, months	*Range*	*(0-22)*	*(0-13)*	*0.038**
	Mean±SD	*11.67±4.6*	*7.5±2.12*	
OS, months	*Range*	*(3-24)*	*(0-15)*	*0.024**
	Mean±SD	*13.22±4.21*	*9.1±3.52*	

NOTE: DFS and OS are given as a median and also at the time point 2 years after diagnosis.

Table 5. *Multivariable analysis for disease-free survival (DFS) and overall survival (OS)*

	DFS		*OS*	
	Hazard ratio (95% CI)	*p-value*	*Hazard ratio (95% CI)*	*p-value*
Wild type CEBPA	*0.066(0.02-0.25)*	*0.001**	*0.125(0.024-0.321)*	*0.002**
High BAALC	*3.98(1.92-5.123)*	*0.003**	*4.215(2.312-6.345)*	*0.001**
Age	*1.012(1-1.17)*	*0.210*	*1.13(0.954-1.52)*	*0.120*
WBCs	*1.002(0.85-1.02)*	*0.531*	*1.1(1.02-1.31)*	*0.212*
Hemoglobin	*0.32(0.22-0.54)*	*0.785*	*0.213(0.13-0.45)*	*0.23*
Platelets	*1.23(1.02-2.34)*	*0.321*	*1.54(1.12-2.17)*	*0.452*
LDH	*1(0.97-1.05)*	*0.890*	*1.02(0.999-1.11)*	*0.321*
% of blood blasts	*0.86(0.53-1.32)*	*0.521*	*0.62(0.32-0.78)*	*0.123*
% of BM blasts	*0.31(0.181-0.734)*	*0.71*	*0.121(0.01-0.46)*	*0.31*

NOTE: Hazard ratios and P values are given for CEBPA mutation versus wild type, and high versus low BAALC expression, as well as for age, WBCs, hemoglobin, platelets, LDH, % of blood blasts and % of bone marrow blasts.

marker in cytogenetically normal AML patients lacking FLT3-ITD and CEBPA mutations. Several lines of evidence suggest that increased expression of BAALC identifies a distinct subset among the leukemic phenotypes. Whereas 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. Furthermore, BAALC expression can be detected in patients with AML and CML in blast crisis, but BAALC transcripts are absent in chronic phase CML and in CLL (19). Given the fact that BAALC expression in normal bone marrow is restricted to the compartment of progenitor cells and that it shows high expression in a subset of leukemic blasts, BAALC may be seen as a stage-specific marker regulated during hematopoiesis and aberrantly expressed in leukemogenesis (33).

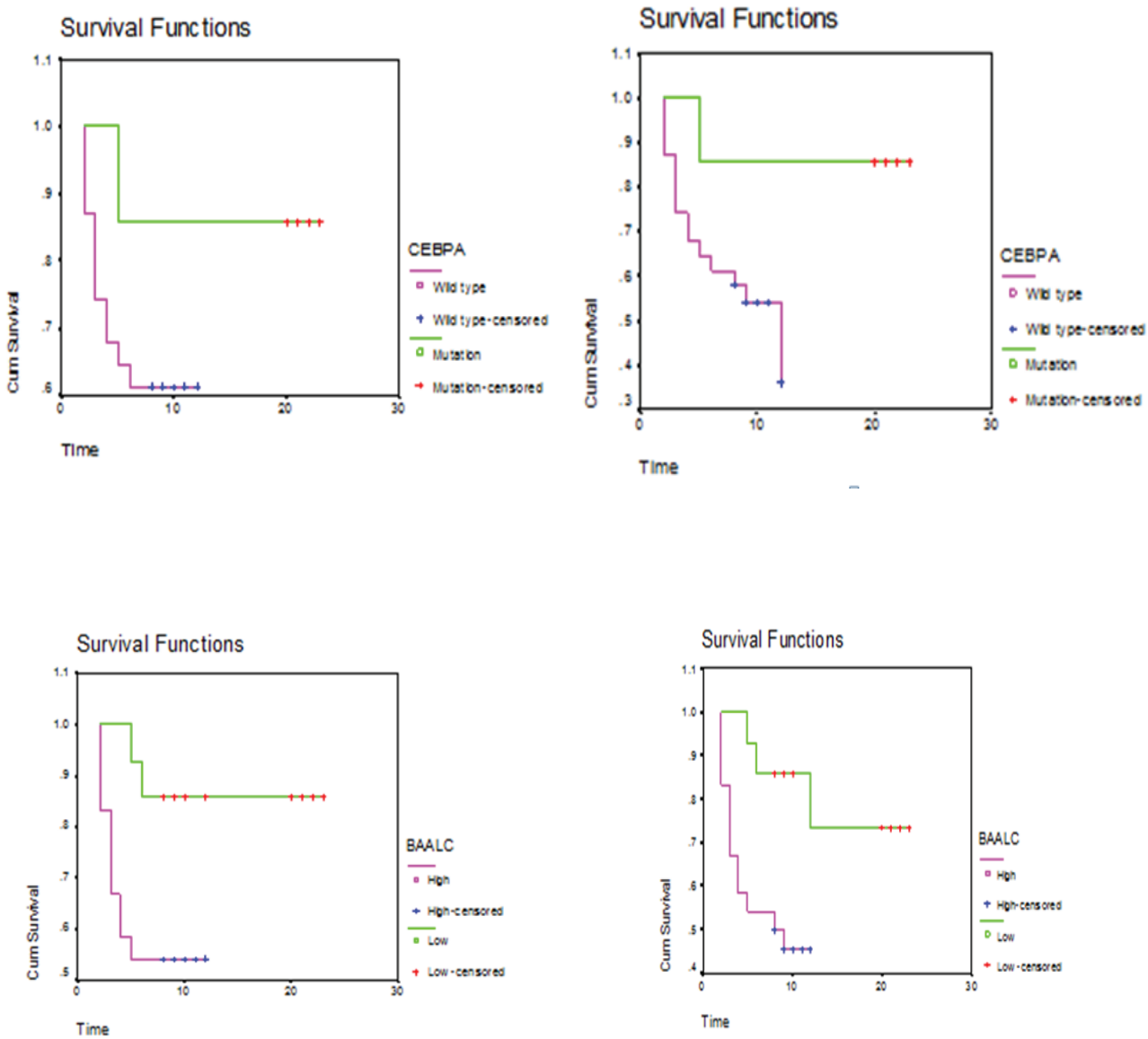

Figure 3. *Kaplan-Meier curves for DFS (A and C) and OS (B and D) in normal-karyotype AML patients according to the CEBPA mutation status (A and B) and the BAALC expression (C and D).*

In summary, we can conclude that identification of CEBPA mutations and differing levels of BAALC expression may have independent prognostic significance in normal-karyotype AML. We propose that molecular assessment of these two factors at diagnosis offers valuable additional prognostic information and may thereby markedly affect therapeutic decisions.

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