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Dysregulation of CCAAT/enhancer binding protein-alpha (*CEBPA*) expression in the bone marrow of acute myeloid leukemia patients

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Abstract

Background: Acute myeloid leukemia (AML) is a heterogeneous malignant disease characterized by accumulation of different types of mutations commonly the CCAAT/enhancer binding protein-alpha (*CEBPA*). However, the dysregulations of *CEBPA* expression in AML is still a debatable issue. The aim of the current study was to assess *CEBPA* gene expression in bone marrow (BM) aspiration specimens of 91 AML patients, compared to 20 control donors of bone marrow transplantation (BMT), using RT-PCR. Data were correlated with patients' clinico-pathological features, response to treatment, progression-free survival (PFS), and overall survival (OS) rates.

Results: There was overexpression of *CEBPA* gene in AML patients compared to normal control [1.7 (0.04–25.6) versus 0.17 (0–4.78), respectively, $P < 0.001$]. Upregulation of *CEBPA* expression associated significantly with increased BM hypercellularity, total leucocyte counts, peripheral blood blast cell count, and poor PFS ($P < 0.001$, 0.002, 0.001, and 0.013, respectively). There was no significant association between *CEBPA* expression and any other relevant clinico-pathological features or OS rates ($P = 0.610$) of the patients. ROC analysis for biological relevance of *CEBPA* expression with AML showed that sensitivity and specificity of *CEBPA* expression at a cut-off value of 0.28 are 92.3% and 78.6%, respectively ($P < 0.001$). All patients who had *CEBPA* overexpression and mutant *FLT3* showed BM hypercellularity, adverse cytogenetic risk, increased TLC, and PB blast cells count ($P = 0.007$, $P < 0.001$, 0.016, and 0.002, respectively).

Conclusion: *CEBPA* overexpression could be used as a genetic biological marker for AML diagnosis, as well as a poor prognostic factor for disease progression. It has no impact on OS rates of the patients.

Keywords: AML, *CEBPA*, *FLT3*, Survival

Background

Acute myeloid leukemia (AML) is a heterogeneous malignant disease of hematopoietic cells that can affect children and adults [1]. It is considered one of the main causes of cancer-related death in children, and the most common acute leukemia in adults [2, 3]. The AML is characterized by accumulation of different types of mutations; one of the most commonly affected mutations is CCAAT/enhancer binding protein-alpha (*CEBPA*) gene [4]. Other reported genes that have been associated with AML development are *fms-related tyrosine kinase 3* (*FLT3-ITD*) and *nucleophosmin 1* (*NMP1*), which play important roles in patients' prognosis and treatment [2]. In addition, Karyotypes of t(15;17), t(8;21), t(16;16) or normal karyotype with double *CEBPA* mutation indicate favorable outcome, whereas −5/5q−, −7/7q−, t(6;9), inv(3), t(9;22), t(v;11q23) complex, and *FLT3* mutation identified patients with high risks and poor outcomes. These patients urgently need intensive therapy especially hematopoietic stem cell transplantation (HSCT) to improve their survival [5]. The choice

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of HSCT is mainly related to the cytogenetic profile of AML patients; however, the advantage of the transplant is exclusive to those who have *CEBPA* mutations associated with other criteria for poor prognosis [6–8]. It has been reported that *CEBPA* mutation is present in approximately 10% of AML patients [9, 10]. In addition, the outcomes of HSCT remain insufficient, and more than 50% of those patients eventually die from the AML malignancy [11].

The *CEBPA* is a transcription factor that affects immune cells density and differentiation [12]. It is a member of the basic region leucine zipper family of transcription factors. This protein is important for the inhibition of self-renewal, cell cycle arrest, and myeloid differentiation during hematopoiesis [13]. The expression of *CEBPA* gene is upregulated during granulocytic differentiation and downregulated during the alternative monocytic pathway [14]. Several studies have reported the association of *CEBPA* expression and the prognosis of AML patients with an intermediate risk karyotype or with distinct cytogenetic risk groups [4, 9, 15].

Although the genetic aberrations and the molecular mechanisms underlying AML had been well known, most patients still had intermediate risk and unfavorable prognosis with poor survival outcomes [16]. Therefore, the aim of the current study was to assess the expression levels of *CEBPA* gene in AML patients from Egypt and evaluate its diagnostic, prognostic, and predictive role(s). The data were correlated to the relevant clinico-pathological features of the patients, response to treatment, progression-free survival (PFS), and overall survival (OS) rates. This may help us to categorize patients according to their clinical response to treatment and also may allow for a new targeted therapy for those patients with *CEBPA* overexpression.

Methods

This is a prospective cohort study included 91 patients with AML, who were presented to the Medical Oncology Department of the National Cancer Institute (NCI) during the period between 2015 to 2016, compared to 20 healthy age- and sex-matched control subjects collected from donors of bone marrow transplantation (BMT). Diagnosis was done according to WHO classification of tumors of the hematopoietic and lymphoid tissues [17]. The cytogenetic risk of the patients was done according to the 2017 European leukemia net (ELN) recommendations [5].

Samples acquisition

All patients and control subjects were presented to the Clinical Pathology Department, NCI, for bone marrow aspiration (BMA). Two drops of BMA were withdrawn to perform smear slides for morphology and cytochemistry. Two BMA specimens were collected from all patients. The first was collected on potassium ethylene diamine tetra-acetic acid (K-EDTA) for immunophenotypic and molecular analyses. The second was collected on sodium heparin for conventional karyotyping and fluorescence in situ hybridization (FISH).

RNA extraction and cDNA formation

BMA samples (1 ml) were obtained from the assessed patients and controls on EDTA anticoagulant tubes. Total RNA was extracted from bone marrow (BM) cells using a QIAamp RNA extraction blood mini kit (QIAGEN® Austin, TX, USA, catalog no. 52304) as recommended by the manufacturer's instructions. The purity and the concentration of the purified RNA was detected using spectrophotometer NanoDrop (Quawell, Q-500, Scribner, USA) and stored at − 80 °C till further assessments. Complementary DNA (cDNA) was prepared using high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA; catalog no. 4368814) according to the manufacturer's instructions. Complementary DNA purity and concentration was assessed and then stored at − 20 °C till performing quantitative real-time PCR.

Real-time PCR (RT-PCR)

The *CEBPA* mRNA expression for enrolled samples was quantified using Taqman Universal PCR Master Mix II (Applied biosystems, USA, Thermo Fisher scientific, Cat no. 4440040) and *CEBPA* Taqman Gene Expression Assay (Applied biosystems, USA, Thermo Fisher scientific, Cat no. 4331182, Hs 00269972-S1). *CEBPA* expression was normalized to *B-actin* as endogenous control. Quantitative real-time PCR (QRT-PCR) was performed using cDNA with the concentration adjusted depending on the abundance of mRNA. The thermal reaction conditions were as follows: 95 °C for 10 min (polymerase activation), followed by 40 cycles of 95 °C for 30 seconds (denaturation) and 60 °C for 60 s (annealing and extension), in which fluorescence was acquired and detected by StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The relative expression of the *CEBPA* gene was analyzed by the comparative Ct method ($2^{-\Delta Ct}$) according to Schmittgen and Livak [18].

Statistical Analysis

Data were analyzed using the SPSS package (version 22 for Windows; SPSS Inc., Chicago, IL, USA). Comparison of gene expression between patients and control was done using Mann-Whitney test. Pearson's χ^2 was used to determine the associations between gene expression and clinico-pathological features of the patients [19]. The area under the receiver operating curve (ROC) was calculated to investigate the best cut-off value, sensitivity, and specificity for diagnosis of AML. Patients were

classified into *CEBPA* gene low and over expression according to the best cut-off value obtained by the ROC curve. Kaplan-Meier was used for comparing survival rates using Log-rank test. Progression-free survival (PFS) was defined as time from date of primary treatment till date of relapse/progressive disease, and overall survival (OS) is the time from date of diagnosis till date of death. *P*-values ≤ 0.05 were considered statistically significant.

Results

Patients' characteristics

The current study included 91 AML patients with a median age of 33 years old, ranged from 18 to 65 years old. Males represented 50/91 (54.9%), and females were 41/91 (45.1%). Patients were classified according to the French-American-British (FAB) classification into M0 (1.72%), M1 (12.1%), M2 (41.8%), M4 (26.4%), M5 (18.7%), and M7 (1.1%). The cytogenetic risk of the patients was as follows: favorable 18 (19.8%), intermediate 51 (56%), and unfavorable 22 (24.2%). BMA of the assessed patients showed hypercellularity in 70 (76.9%), hypo-cellularity in 7 (7.7%), and normo-cellularity in14 (15.4%) patients. Lymphadenopathy was detected in 30 (33.0%) patients, hepatomegaly in 27 (29.7%), and splenomegaly in 23 (25.3%). Fourteen patients (15.4%) had *FLT3* ITD mutation and 77 (84.6%) had wild type *FLT3*. The other clinico-pathological features were summarized in Table 1.

Expression levels of *CEBPA* gene in the patients' groups

The median expression and range of *CEBPA* in AML patients was 1.7 (0.04–25.6), while it was 0.17 (0–4.78) in the normal control group. The difference in the expression between the two groups was statistically significant ($P < 0.001$, Fig. 1a).

ROC analysis was performed to assess the biologic relevance of *CEBPA* expression for AML patients against the control group. It showed 92.3% sensitivity and 78.6% specificity at a cut-off value of 0.28 with area under curve (AUC) 0.826 ($P < 0.001$, Fig. 1b). It was noted that all patients with *FLT3-ITD* mutation had *CEBPA* expression over the cut-off value of 0.28 (Fig. 1c).

Association between *CEBPA* expression and clinico-pathological features of the patients

Patients were classified according to the median into 44 (48.4%) patients with low CEBPA expression (< 1.7), and 47 (51.6%) patients with *CEBPA* overexpression (> 1.7). There was a significant increase in the total leucocyte count (TLC) in patients with *CEBPA* overexpression [61 (1–440) × 10^9/L], compared to those with *CEBPA* low expression [16 (2.2–240) × 10^9/L, $P = 0.002$]. Also, the percentage of peripheral blood blasts was significantly increased in patients with *CEBPA* overexpression, compared to those with *CEBPA* low expression [70 (0–98) versus 40 (0–95), respectively, $P = 0.001$]. Meanwhile, the percentage of bone marrow blasts was increased in patients with *CEBPA* overexpression, compared to those with *CEBPA* low expression [75 (33–97) versus 65 (14–95), respectively]; however, this association is nearly significant ($P = 0.051$). Patients with *CEBPA* overexpression showed significant BM hypercellularity ($P < 0.001$), since out of all patients who had *CEBPA* over expression, there were 44/47 (93.6%) that showed hypercellular BM, 2/47 (4.3%) showed normocelluar BM, and only 1/47 patient (2.1%) had hypocellular BM. However, there was no significant association between *CEBPA* expression and any of the other relevant clinico-pathological features of the patients ($P > 0.05$, Table 2).

Patients who had both *CEBPA* overexpression and mutant *FLT3* showed BM hypercellularity, adverse cytogenetic risk, and increased TLC and PB blast cells count. Since, BM hypercellularity was present in 88.9% (8/9) of patients with *CEBPA* overexpression and mutant *FLT3*, 94.7% (36/38) of patients with *CEBPA* overexpression and wild type *FLT3*, 60% (3/5) in patients with *CEBPA* low expression and mutant *FLT3*, compared to 59% (23/39) in patients with *CEBPA* low expression and wild *FLT3* ($P = 0.007$, Fig. 2a). Also, all patients with (*CEBPA* overexpression and mutant *FLT3*) and those with (*CEBPA* low expression and mutant *FLT3*) were presented with adverse cytogenetic risk (100% for both groups), compared to 12.8% of patients with *CEBPA* low expression and wild *FLT3*, or 7.9% of patients with *CEBPA* overexpression and wild *FLT3* ($P < 0.001$, Fig. 2b). Patients with *CEBPA* overexpression and mutant *FLT3* had a significantly increased TLC (115, range; 1–440 × 10^9/L), compared to those with *CEBPA* low expression and wild *FLT3* (16.4, range; 2–403 × 10^9/L, $P = 0.016$, Fig. 2c). On the other side, patients with *CEBPA* low expression and wild *FLT3* showed a significant decrease in PB blast cell count (32.5%, range; 0–95%), compared to the other patients' groups ($P = 0.002$, Fig. 2d).

Overall Survival (OS) and progression-free survival (PFS) rates of the patients

Out of the 91 assessed AML patients, there were 68 (74.7%) who had complete remission (CR), and 23 (25.3%) patients had progressive disease. There was a significant association between PFS rate and *CEBPA* expression, since the median PFS time for patients with *CEBPA* low expression was 40.7 months compared to 12.4 months for patients with *CEBPA* overexpression ($P = 0.013$, Fig. 3a).

During the follow-up period of the patients, there were 54 (59.3%) that died and 37 (40.7%) alive. There was no

Table 1 Clinico-pathological features of the assessed AML patients

Patients' characteristics	Frequency (%)
Age (median and range), years	33 (18–65)
TLC (median and range), × 10^9/L	38.9 (1–440)
HB (median and range), g/dl	8 (5.5–13)
Sex	
Male	50 (54.9)
Female	41 (45.1)
BM cellularity	
Hypercellular	70 (76.9)
Hypocellular	7 (7.7)
Normocellular	14 (15.4)
FAB classification	
M1	11 (12.1)
M2	38 (41.8)
M4	24 (26.4)
M5	17 (18.7)
M7	1 (1.1)
Organomegally	
Hepatomegaly	27 (29.7)
Spleenomegaly	23 (25.3)
CD34	
Negative	27 (29.7)
Positive	64 (70.3)
CD13	
Positive	91 (100)
CD117	
Negative	19 (20.9)
Positive	72 (79.1)
CD14	
Negative	47 (51.6)
Positive	44 (48.4)
Aberrant IPT markers	
Without aberrant marker	73 (80.2)
With aberrant marker	18 (19.8)
Response to treatment	
CR	68 (74.7)
No CR	23 (25.3)
PLT (median and range)×10^9/L	35 (5–297)
BP blast (median and range) %	50 (0–98)
BM blast (median and range) %	70 (14–97)
CD4	
Negative	54 (59.3)
Positive	37 (40.7)
Cytogenetic risk	
Favorable	18 (19.8)
Intermediate	51 (56.0)
Unfavorable	22 (24.2)
IPT	
Mono	7 (8.0)
Myelo	50 (57.5)
Myelomono	29 (33.3)
Megakaryoblastic	1 (1.1)
Lymphadenopathy	
Negative	61 (67.0)
Positive	30 (33.0)
MPO	
Negative	3 (3.3)
Positive	88 (96.7)
CD33	
Positive	91 (100)
HLA/DR	
Negative	16 (17.6)
Positive	75 (82.4)
CD11c	
Negative	52 (57.1)
Positive	39 (42.9)
FLT3 (ITD)	
Wild	77 (84.6)
Mutant	14 (15.4)
Death	
Alive	37 (40.7)
Dead	54 (59.3)

significant association between the OS rates of the patients and *CEBPA* expression, since the median OS time for patients with *CEBPA* low expression was 9 months compared to 11.4 months for patients with *CEBPA* overexpression (P = 0.610, Fig. 3b).

On the other hand, combined CEBPA overexpression with *FLT3* mutation had no significant impact on PFS or OS rates (P = 0.081 and P = 0.664, respectively, Fig. 3c, d)

Discussion

Different chromosomal abnormalities and genomic alterations had been reported to play important roles in the pathogenesis of AML, especially recurrent mutations in the *FLT3*, *NPM1*, *DNMT3A*, and *IDH1* [20]. However, many patients with AML have no mutations in any of the currently recognized genes associated with the

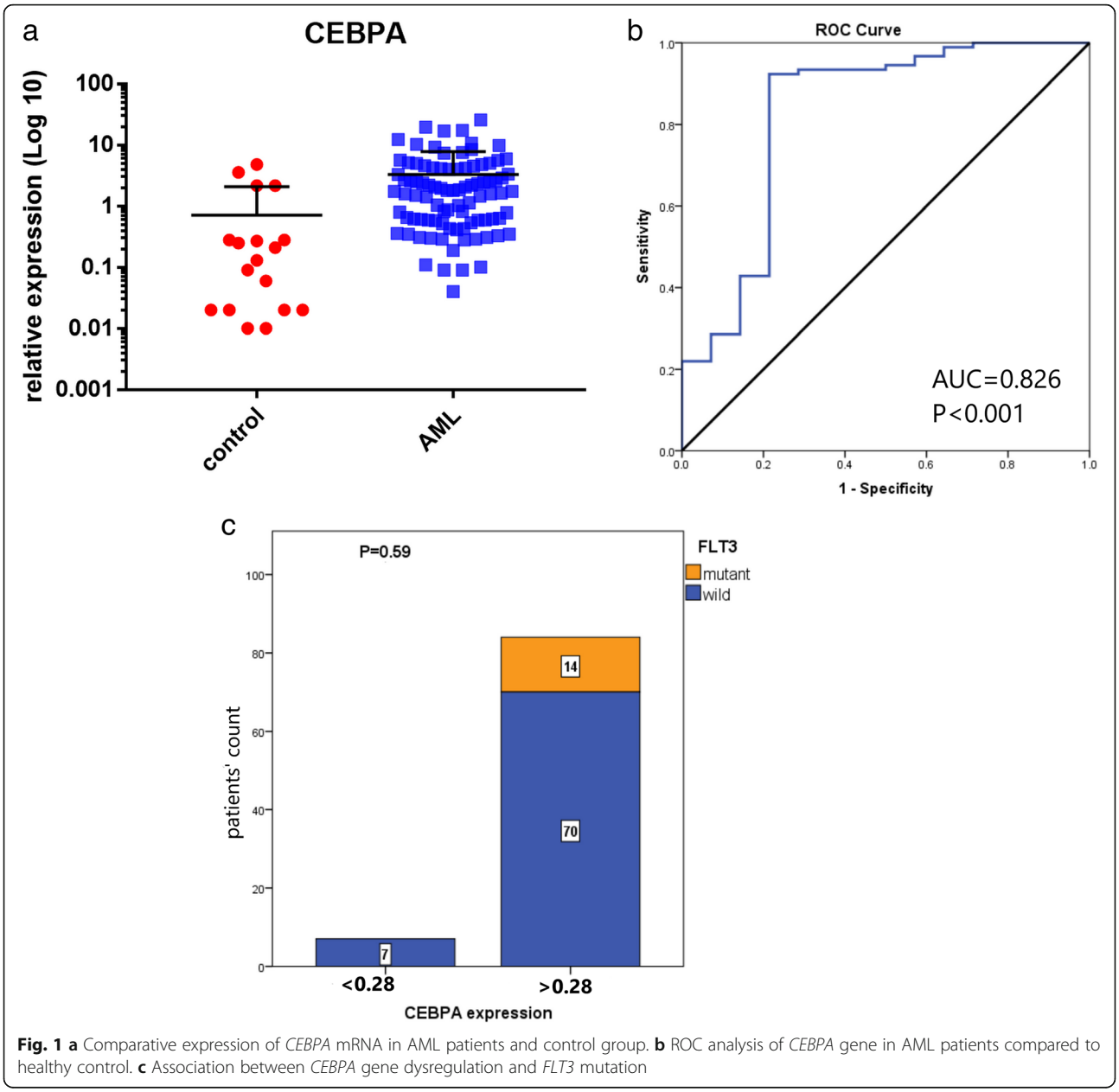

Fig. 1 **a** Comparative expression of *CEBPA* mRNA in AML patients and control group. **b** ROC analysis of *CEBPA* gene in AML patients compared to healthy control. **c** Association between *CEBPA* gene dysregulation and *FLT3* mutation

progression of AML [21]. So it is essential to investigate other molecular aberrations that may affect the pathogenesis of AML or can predict patients' outcomes. The aim of the current study was to assess the expression level of *CEBPA* gene in AML patients, regarding its biological role and prognostic and predictive value(s).

The reported data in literature regarding *CEBPA* expression levels in AML patients is still controversial. Our results demonstrated a significant overexpression of *CEBPA* in AML patients compared to normal control, which confirm that this gene upregulation may have a role in the AML pathogenesis. These data are consistent with many recent published studies that reported upregulation of *CEBPA* in AML patients [15, 22, 23]. Mustafa et al. also determined five pathogenic *CEBPA* mutations using bioinformatics analysis, which could be used as genetic biomarkers for AML [11]. On the other hand, several studies reported contradictory results regarding this issue; they demonstrated that there were no significant alterations in *CEBPA* expression in AML patients [24–26]. This discrepancy in results may be due to the variability in the genetic makeup and the underlying etiological factors for mutations between different populations, different clinical features, sample size, and different assessment methods among studies.

Table 2 Association between CEBPA gene expression and patients' characteristics

	CEBPA expression		*P* value
	Low expression (44)	Overexpression (47)	
Age (years)	33 (18–65)	34 (18–57)	0.499
TLC ($\times 10^9$/L)	16 (2.2–240)	61 (1–440)	**0.002**
Platelet count ($\times 10^9$/L)	35 (7–110)	34 (5–297)	0.799
HB (gm/dl)	7.9 (4.6–12.1)	8 (5.5–13)	0.859
PB blast (%)	40 (0–95)	70 (0–98)	**0.001**
BM blast (%)	65 (14–95)	75 (33–97)	**0.051**
Gender			
Male	21 (47.7%)	29 (61.7%)	0.21
Female	23 (52.3%)	18 (38.3%)	
BM cellularity			
Hypercellular	62 (59.1%)	44 (93.6%)	***P* < 0.001**
Hypocellular	6 (13.6%)	1 (2.1%)	
Normocellular	12 (27.3%)	2 (4.3%)	
FAB			
M1	5 (11.4%)	6 (12.8%)	0.77
M2	18 (40.9%)	20 (42.6%)	
M4	13 (29.3%)	11 (23.4%)	
M5	7 (15.9%)	10 (21.3%)	
M7	1 (2.3%)	0 (0.0%)	
FLT3-ITD			
Wild	39 (88.6%)	38 (80.9%)	0.39
Mutant	5 (11.4%)	9 (19.1%)	
Cytogenetics			
Abnormal	32 (72.7%)	35 (74.5%)	0.85
Normal	12 (27.3%)	12 (25.5%)	
Cytogenetic risk			
Adverse	10 (22.7%)	12 (25.5%)	0.48
Intermediate	23 (52.3%)	28 (59.6%)	
Favorable	11 (25.0%)	7 (14.9%)	
Hepatomegaly			
Negative	28 (63.6%)	36 (76.6%)	0.25
Positive	16 (36.4%)	11 (23.4%)	
Spleenomegaly			
Negative	31 (70.5%)	37 (78.7%)	0.47
Positive	13 (29.5%)	10 (21.3%)	
Lymphadenopathy			
Negative	31 (70.5%)	30 (63.8%)	0.51
Positive	13 (29.5%)	17 (36.2%)	
CD34			
Negative	14 (31.8%)	13 (27.7%)	0.82
Positive	30 (68.2%)	34 (72.3%)	
CD117			
Negative	13 (29.5%)	6 (12.8%)	0.07
Positive	31 (70.5%)	41 (87.2%)	

Table 2 Association between CEBPA gene expression and patients' characteristics *(Continued)*

	CEBPA expression		*P* value
	Low expression (44)	Overexpression (47)	
HLA/DR			
Negative	7 (15.9%)	9 (19.1%)	0.786
Positive	37 (84.1%)	38 (80.9%)	
CD4			
Negative	27 (61.4%)	27 (57.4%)	0.831
Positive	17 (38.6%)	20 (42.6%)	
CD14			
Negative	23 (52.3%)	24 (51.1%)	0.908
Positive	21 (47.7%)	23 (48.9%)	
CD11c			
Negative	24 (54.5%)	28 (59.6%)	0.675
Positive	20 (45.5%)	19 (40.4%)	
IPT			
Mono	2 (4.9%)	5 (10.9%)	0.386
Myelo	22 (53.7%)	28 (60.9%)	
Myelomono	16 (39.0%)	13 (28.3%)	
Megakaryoblastic	1 (2.4%)	0 (0.0%)	
Aberrant IPT markers			
Negative	38 (86.4%)	35 (74.5%)	0.193
Positive	6 (13.6%)	12 (25.5%)	

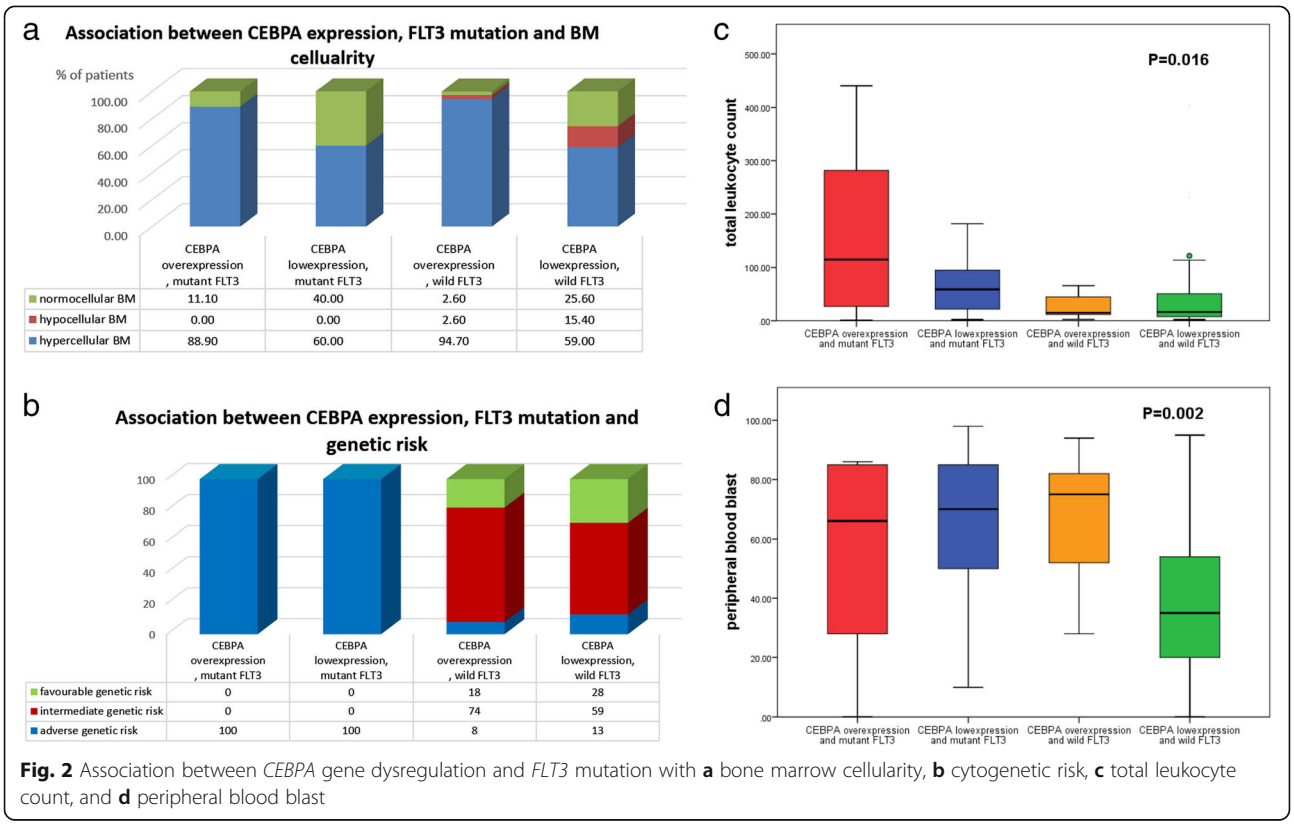

Fig. 2 Association between *CEBPA* gene dysregulation and *FLT3* mutation with **a** bone marrow cellularity, **b** cytogenetic risk, **c** total leukocyte count, and **d** peripheral blood blast

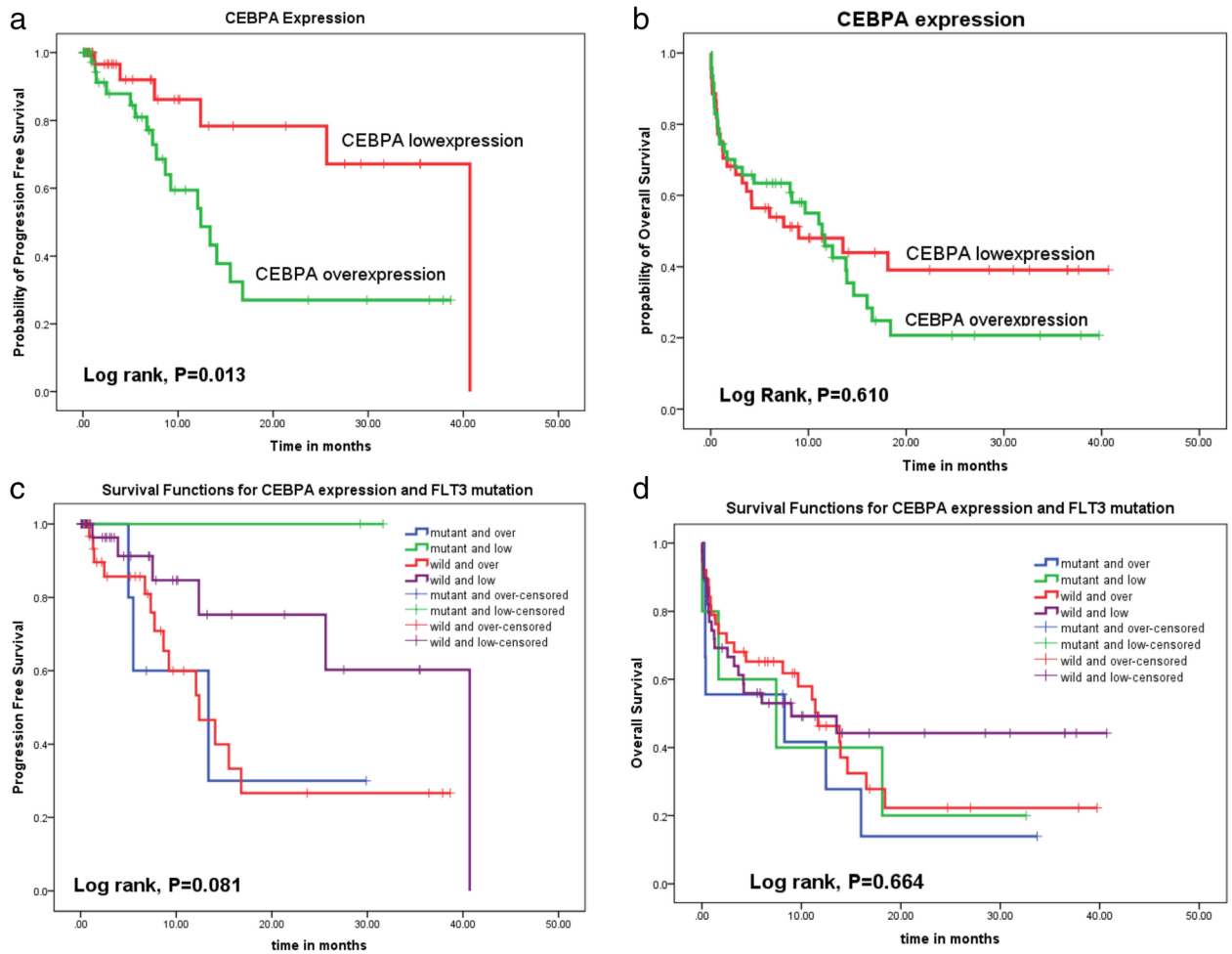

Fig. 3 Impact of *CEBPA* gene expression on **a** PFS and **b** OS. Impact of *CEBPA* overexpression and *FLT3* mutation on **c** disease-free survival and **d** overall survival rates

The current study demonstrated that patients with *CEBPA* overexpression showed a significantly increased total leucocyte count (TLC) and bone marrow hypercellularity, as well as increased percentage of peripheral blood blast cells in the patients. These data are comparable to that reported by D'Alò et al., who demonstrated that low *CEBPA* expression was observed in patients with leukopenia and decreased expression of CD33 and CD11c antigens at diagnosis [25]. Meanwhile, we could not find a significant association between *CEBPA* expression and any of the other clinical characteristics assessed including age, sex, cytogenetic risk classification, abnormal karyotyping, organomegaly, lymphadenopathy, and FAB classification. These results are supported by Doorn et al., who reported that *CEBPA* expression levels had no association with patients' clinical features [15]. Similarly, Salarpour et al. demonstrated that there was no significant association between *CEBPA* expression and FAB subgroups, which indicates that *CEBPA* expression level is not dependent on the type of lineage involved (granulocytic vs. monocytic) or levels of cell maturation [23]. However, Gholami et al. observed a significant up-regulation of *CEBPA* in the male AML patients, abnormal karyotype, and FAB subtypes (M0, M3, and M4), as well as in favorable and adverse cytogenetic risk groups [16].

According to our results, there was a significant association between *CEBPA* expression and progression-free survival rate of the assessed AML patients, while there was no significant association with the overall survival rate. This data is comparable to that of Doorn et al., who observed that *CEBPA* expression levels had no impact on PFS or OS rates of AML patients, while patients with *CEBPA* gene C-terminal mutations and frame shift mutations in the N-terminus, showed longer event-free survival (EFS) and OS rates than patients lacking these mutations [15].

Additionally, we found that *FLT3-ITD* mutation was present in 15.4% of the assessed patients; this frequency

is in concordance with 19.8% *FLT3-ITD* mutation reported by Wang et al. [27]. An interesting finding in the present study is that all patients who had both *CEBPA* overexpression and mutant *FLT3* showed poor outcomes, since all of them had adverse cytogenetic risk, BM hypercellularity, increased TLC, and PB blast cells count. This is consistent with other published studies reported that *FLT3-ITD* mutations is a poor prognostic factor associated with refractory disease, increased relapse risk, and poor OS [28–30].

Therefore, the current study provides evidence that CEBPA overexpression may play a role in the progression of AML, though its main function is critically involved in myeloid differentiation; however, our results demonstrated its upregulation in AML. Thus, it is suggested that *CEBPA* gene is involved in cellular proliferation machinery rather than differentiation during the process of tumorigenesis [23]. Meanwhile, one of the drawbacks in this study is that the assessment of *CEBPA* gene expression would be helpful if performed in the isolated CD34+ stem/progenitor cells from healthy controls. However, due to the difficulty of obtaining the normal BM samples (donors for BM transplantation), and also the decreasing percentage of CD34+ stem cells (< 5%), hence, we rely on total BM cells isolated from healthy control subjects.

Conclusion

We can conclude that *CEBPA* overexpression could be considered a diagnostic biological marker for AML patients, as well as a poor prognostic factor for disease progression. It has no impact on OS rates of the patients. However, further studies are required on a larger number of patients to assess more deeply the role of its expression dysregulation in AML, and the affected pathways involved in this process. This will open a new avenue for evolving new modalities of treatment targeted to this affected genes and thereby produce better clinical outcomes for those AML patients with *CEBPA* dysregulation.

Abbreviations

AML: Acute myeloid leukemia; BM: Bone marrow; BMT: Bone marrow transplantation; *CEBPA*: CCAAT/enhancer binding protein-alpha; EFS: Event-free survival; ELN: European leukemia net; FAB: French-American-British; FISH: Fluorescence in situ hybridization; *FLT3-ITD*: Fms-related tyrosine kinase 3; HSCT: Hematopoietic stem cell transplantation; K-EDTA: Potassium ethylene diamine tetra-acetic acid; *NMP1*: Nucleophosmin 1; OS: Overall survival; PFS: Progression-free survival

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Authors' contributions

NH put the idea and study design and supervised the work. *FS* performed the cytogenetics and shared in flow cytometry work and also follow-up of the patients. *RS* collected data and performed the flow cytometry and molecular work. *MS* shared in the molecular work and performed the statistical analysis and drafting the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The research protocol was approved by the Institutional Review Board Ethical Committee of the National Cancer Institute [201617027-4], which follows the rules of the Helsinki Institutional Review Board.
A written informed consent was obtained from all patients and healthy individuals before enrolment in the study.

Consent for publication

All participants agreed for publication.

Competing interests

All authors declare no competing interest.

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