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ORIGINAL RESEARCH

Impact of *HOXB4* and *PRDM16* Gene Expressions on Prognosis and Treatment Response in Acute Myeloid Leukemia Patients

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Introduction: Acute myeloid leukemia (AML) is the most common type of leukemia among adults and is characterized by various genetic abnormalities. *HOXB4* and *PRDM16* are promising markers of AML. Our objective is to assess the potential roles of *HOXB4* and *PRDM16* as prognostic and predictive markers in newly diagnosed AML patients and determine the correlation between their expressions and other prognostic markers as *FLT3-ITD, NPM1 exon 12* mutations, response to treatment, and patient's survival.

Methods: This study included 83 de novo AML adult patients. All patients were subjected to clinical, morphological, cytochemical, and molecular analysis to detect *HOXB4* and *PRDM16* gene expressions and *FLT3-ITD, NPM1 exon 12* mutations.

Results: The results showed that a low expression of *HOXB4* was found in 31.3% of AML patients, whereas a high expression of *PRDM16* was evident in 33.8% of AML patients. *FLT3-ITD* mutations were detected in 6 patients (7.2%), while *NPM1 exon 12* mutations were detected in 7 patients (19.4%) out of 36 patients with intermediate genetic risk. Out of the 50 patients who achieved complete remission (CR), relapse occurred in 16% of the cases. Low expression of *HOXB4* and high expression of *PRDM16* were associated with CR of 32% and 28%, respectively, and a short overall survival (OS) and disease-free survival (DFS).

Conclusion: Further larger study should be conducted to verify that high *PRDM16* and low *HOXB4* gene expressions could be used as a poor prognostic predictor for AML. The correlation between *PRDM16* and *HOXB4* gene expressions and *FLT3-ITD* and *NPM1 exon 12* mutations might have a role on CR, relapse, OS, and, however, this should be clarified in analysis with a larger number of samples.

Keywords: AML, HOXB4, PRDM16, NPM1 exon 12, FLT3-ITD

Introduction

Acute myeloid leukemia (AML), the most prevalent myeloid malignancy in adults, is characterized by genetic and epigenetic abnormalities in hematopoietic progenitors that result in dysregulation of critical processes, including proliferation self-renewal, and terminal differentiation.[1,2] It is responsible for 15–20% of acute childhood leukemia.[3] AML is diverse both across individuals and within the same patient, with varying clinical symptoms, molecular abnormalities, cytogenetics, and responsiveness to therapy.[4] AML has been divided into eight French-American-British (FAB) subgroups based on morphologic-genetic heterogeneity.[5]

The use of the newly approved treatment approaches to personalize therapy and improve outcomes in AML patients has progressed since 2017.[6] Advances in genome-wide molecular profiling and immunophenotyping (IPT) have identified mutations in genes associated with apoptosis (p53, nucleoplasmin, etc.) and regulation of cell proliferation (RAS, Fms-like tyrosine kinase 3, c-KIT, etc.) as possible prognostic biomarkers in AML. However, targeting these factors has failed to tackle the increasing disease heterogeneity and outcome, limiting personalized approaches for AML

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patients. The average 2-yr and 5-yr overall survival (OS) rates of patients diagnosed with AML are approximately 32% and 27%, respectively.[7,8] Thus, there is an urgent need for novel prognostic and predictive genetic biomarkers to guide patient-tailored treatment and improve survival outcomes.

The homeobox (HOX) family of transcription factors is required for normal anatomical development. *HOXB4* is a positive regulator of hematopoietic stem cell self-renewal that has been classified as a tumor suppressor or oncogene depending on the kind of cancer. *HOXB4* overexpression is required for the development and progression of several forms of cancer, including lung,[9] ovarian,[10] bladder, renal,[11] mesothelioma,[12] and leukemia.[13,14] Additionally, elevated *HOXB4* expression is related to a poor prognosis for malignant mesothelioma.[12] Other investigations have shown downregulation of the *HOXB4* gene in cancer tissues.[15,16]

PRDM16 is a member of the PRDI-BF1 and RIZ domain-containing protein families. It is structurally distinct from the others by having a conserved N-terminal PR domain and a variable number of zinc fingers.[17,18] *PRDM16* has intrinsic histone methyltransferase activity, allowing it to catalyze histone-3 lysine methylation (H3K9me1).[19] As a result, *PRDM16* may also participate as a transcriptional regulator, either directly or indirectly, via complex formation with histone-modifying enzymes.[20] *PRDM16* is required to maintain hematopoietic stem cells,[21] which makes it an attractive potential gene for leukemogenesis induction.[22] While new research suggests that *PRDM16* may contribute to the prediction of poor outcomes in juvenile AML patients,[23] the prognostic importance of *PRDM16* remains uncertain.

Around 30% of AML patients display the *FLT3-ITD* mutation. Patients with this mutation have a poor prognosis. Early detection of *FLT3-ITD* may allow for more sustained and permanent remissions.[24] Additionally, previous articles revealed that mutations in Nucleophosmin 1 (*NPM1*) *exon 12* may have prognostic importance in Egyptian AML patients, providing vital new prognostic information and potentially significantly affecting therapy choices.[25,26]

The current study evaluated the potential prognostic and predictive roles of *HOXB4* and *PRDM16* in newly diagnosed AML patients and established a correlation between their expression and other prognostic factors such as cytogenetic abnormalities, *FLT3-ITD, NPM1 exon 12* mutations, response to treatment, and patient survival.

Methods

Subjects and Samples

This study was conducted at the National Cancer Institute (NCI), Cairo University, Egypt, and included 83 newly diagnosed AML adult patients (median 40 years) referred to Medical Oncology Department between January 2018 and June 2021.

The inclusion criteria were that the patient had to be newly diagnosed with AML and have no prior treatment history. Exclusion criteria included being a secondary AML patient, having significantly compromised hepatic or renal function, having concomitant severe or uncontrolled medical problems (eg, uncontrolled diabetes, infection, or hypertension), or having a family history of hematological malignancies.

Ten apparently healthy persons (age and sex-matched) who had bone marrow (BM) aspiration for reasons other than malignancy served as normal controls.

All participants provided written informed consent. The research was approved by the institutional review board of the National Cancer Institute, Cairo University, Cairo, Egypt, following the Helsinki Declaration and its recent amendments.

Clinical, cytomorphological, cytogenetic, and molecular analyses of BM samples were used to diagnose all patients. The European LeukemiaNet-2017 (ELN-2017) genetic risk categorization system was used to classify AML patients.[27] All patients were diagnosed with AML using the FAB and WHO criteria.[28]

Treatment Protocol and Follow-Up

In general, all patients were given the standard front-line (3+7) IA/DA-like induction regimens consisting of idarubicin/daunorubicin for three days (10/45 mg/m^2, Day 1–3) and cytarabine for seven days (100 mg/m^2, Day 1–7).

Complete remission (CR) was described as when less than 5% of leukemia blasts remain in the BM; extramedullary disorders were absent, neutrophil counts >1 x 10^9/L, and counting of platelets >100 x 10^9/L in the peripheral blood. Following CR, the consolidation was achieved by four cycles of high-dose cytarabine (2 g/m^2).

All patients were followed until June 2021. The OS was calculated from the date of AML diagnosis to the date of death and was censored at the time of the final follow-up. Patients who underwent hematopoietic stem cell transplantation (HSCT) were censored at the start of the procedure. DFS was estimated from the date of initial diagnosis to the date of relapse or death from any cause, whichever occurred first.

Sampling and Laboratory Work-Up

All patients had the following laboratory evaluations: peripheral blood examination (CBC: hemoglobin (Hb) level, total leukocyte count (TLC), platelet count, and blast cell percentage), bone marrow examination, IPT, and cytogenetic analysis.

Two drops of BM aspirate specimens were collected from all patients. The first was collected on K-EDTA for IPT and molecular analyses, and the second was collected on sodium heparin for conventional karyotyping and fluorescence in situ hybridization (FISH). Two BM aspirates were withdrawn to perform smear slides for morphology and cytochemistry.

Quantitative Real-Time PCR (qRT-PCR) of *PRDM16* and *HOXB4* mRNA

Total RNA was extracted from BM cells according to the manufacturer's recommendations using a QIAamp RNA extraction blood micro kit (QIAGEN® Austin, TX, USA, catalog no. 52304). The purity and concentration of extracted RNA were determined using a spectrophotometer NanoDrop (Quawell, Q-500, Scribner, USA) and the samples were kept at −80 °C until further evaluation.

Complementary DNA (cDNA) was produced according to the manufacturer's instructions using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA; catalog no. 4368814). The purity and concentration of complementary DNA were determined and then kept at −20 °C until qRT-PCR was performed.

The expression of *PRDM16* and *HOXB4* mRNA in enrolled samples was evaluated using TaqMan Universal PCR Master Mix II (Applied Biosystems, USA; Thermo Fisher Scientific, Cat. no. 4440040) and the *PRDM16* and *HOXB4* TaqMan Gene Expression Assay (Applied biosystems, USA, Thermo Fisher Scientific, Cat no 4453320, Hs 00223161-m1, Hs 00256884-m1). The expression of *PRDM16* and *HOXB4* was normalized to the endogenous control β-actin. 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 minutes (polymerase activation), followed by 40 cycles of 95 °C for 30 seconds (denaturation) and 60 °C for 60 seconds (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 *HOXB4* and *PRDM16* gene expression was assessed relative to the housekeeping gene using the $2^{-\Delta\Delta Ct}$ method.[29] The data were expressed as the fold change in *HOXB4* and *PRDM16* gene expression in patients relative to healthy controls and normalized to the expression levels of the endogenous control.

Analysis of FLT3 Gene Mutations

According to the manufacturer's protocol, high molecular weight DNA was extracted from BM/EDTA samples using QIAamp DNA Blood Mini Kit (QIAGEN). PCR amplification was carried out using *FLT3-ITD* master mix containing dNTPs, the forward (5′-CAATTTAGGTATGAAAGCC-3′) and reverse (5′-GTACCTTTCAGCATTTTGAC-3′) primers (Invivoscribe Technologies, Inc., USA). Positive and negative control DNAs (Invivoscribe Technologies, Inc., USA), AmpliTaq DNA polymerase (Applied Biosystems, Life Technologies, USA), and 100 bp DNA ladder (Invitrogen, Life Technologies, USA) were used.

In brief, 1 µL DNA was amplified in a volume of 25 µL containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 Mm $MgCl_2$, 200 mM dNTPs, 0.5 µM of each primer, and 1 U Taq DNA polymerase (QIAGEN). The PCR consisted of an initial incubation step at 94 °C for 150 seconds followed by 35 cycles at 94 °C for 30 seconds, 57 °C for 60 seconds, 72 °C for 120 seconds, and a final elongation step at 94 °C for 30 seconds, and 60 °C for 10 minutes. The PCR product was

analyzed on standard 3% agarose gel stained with ethidium bromide. A fragment of 328 base pairs (bp) was produced from wild-type (WT) alleles. All patients with an additional higher molecular weight band were considered *FLT3-ITD*+.

Analysis of *NPM1 Exon 12* Mutations

Patients with intermediate genetic risk (normal cytogenetic results) were selected for molecular analysis of *NPM1 exon 12* mutations.

Genomic DNA was extracted from BM/EDTA samples using QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's protocol. For *NPM1* mutation analysis, *NPM1 exon 12* was amplified by genomic PCR using primers NPMex12F/ CTGATGTCTATGAAGTGTTGTGGTTCC (sense) and NPMex12R/ CTCTGCATTATAAAAAGG ACAGCCAG (antisense). The reaction mixture was made up of 50 µL of the following constituents: 100 ng of genomic DNA, 0.5 U Taq DNA polymerase, 1X Taq polymerase buffer, 1.75 mM $MgCl_2$, 0.4 µM *NPM1* primers, and 0.4 mM dNTP. The samples were amplified by initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 53 °C for 1 minute, 72 °C for 2 minutes, and final extension at 72 °C for 10 minutes. They were checked on 2% agarose gel electrophoresis using a DNA marker.

PCR products were mixed with ten volumes of loading buffer, denatured at 96 °C for 5 minutes, quenched on ice immediately, and applied to 10% polyacrylamide gel electrophoresis. Normal *NPM1* exhibits a specific conformational pattern. A mutant gene displays a pattern with different electrophoretic mobility (mobility shift)

Statistical Analysis

The statistical analyses were carried out using the IBM SPSS (SPSS for Windows release, version 22.0, SPSS, Chicago, IL, USA). Continuous variables were expressed as mean and standard deviation or median and range, whichever is appropriate. To represent categorical variables, the frequency and percentage were used. Chi-square and Fisher's exact test investigated the correlation between qualitative variables. The Kruskal–Wallis test (non-parametric ANOVA) compared three groups, followed by post-hoc pair-wise comparisons. Mann–Whitney test was used to compare two groups. The Kaplan-Meier method was used for survival analysis, and the Log rank test was used to compare the two survival curves. The tests were two-tailed, and a p-value <0.05 was deemed significant.

Results

Patient's Characteristics

Table 1 shows the baseline patient characteristics. The median age at diagnosis for the entire study cohort was 40 (range, 18–57) years, with 46 males (55.4%) and 37 females (44.6%) present. BM hypercellularity was found in 62 patients (74.7%). AML-M2 was the most frequent FAB subtype representing 39.8% of the patients, followed by M4 and M1 subtypes. Sixteen patients (19.3%) were classified as high-risk, 36 (43.4%) were intermediate-risk, and 31 (37.3%) were low-risk. Mutational analysis has shown that six patients (7.2%) had *FLT3-ITD* mutations while seven patients (19.4%) had *NPM1 exon 12* mutations.

Expression of *HOXB4* and *PRDM16* in AML Patients

Figure 1 shows that the mean fold change of *HOXB4* and *PRDM16* gene expressions were significantly higher in AML (23.49 and 17.36, respectively) compared to the control (0.94 and 1.16, respectively; $p < 0.001$). The *HOXB4* and *PRDM16*mRNA expression was classified into two categories (low vs high) according to the median of the *HOXB4* and *PRDM16* gene expression (3.21 and 0.67, respectively). High expression of *HOXB4* was found in 68.67% (57/83) of AML patients, whereas high expression of *PRDM16* was evident in 33.73% (28/83) of AML patients, as shown in Table 2.

Relations Between *HOXB4* and *PRDM16* Expression and Patient's Characteristics

No significant associations were found between *HOXB4* and *PRDM16* expression and patient characteristics except for the significant-high *HOXB4* expression in the male group (36/46, 78.3%) as compared to the female group (21/37,

Table 1 Demographic Data of All Studied Patients

Variables		N= 83	%
Age: (years)*		40.0 (18–57)	
Gender	Male	46	55.4
	Female	37	44.6
TLC x10^9 /mm^3*		30.0 (0.5–616.0)	
Hb (gm/dl) *		7.7 (3.7–13.2)	
Platelets x10^9 /mm^3*		36.0 (5.0–826.0)	
Peripheral blood blasts %*		42.0 (0.0–96.0)	
BM blasts %*		60.0 (20.0–97.0)	
BM cellularity	Normocellular	17	20.5
	Hypercellular	62	74.7
	Hypocellular	4	4.8
FAB	M0	2	2.4
	M1	12	14.5
	M2	33	39.8
	M3	2	2.4
	M4	25	30.1
	M5	6	7.2
	M7	3	3.6
IPT	Myeloid	49	59.0
	Monocytic	6	7.2
	Myelomonocytic	25	30.1
	Megakaryoblastic	3	3.6
Genetic risk	Low	31	37.3
	Intermediate	36	43.4
	High	16	19.3
FLT3-ITD	Wild	77	92.8
	Mutant	6	7.2
NPM1 exon 12 (N=36)	Wild	29	80.6
	Mutant	7	19.4

Note: *Median (Min-Max).

56.8%) (p=0.036) and high *PRDM16* among patients with hypocellular BM (4/4, 100%) as compared to those with hypercellular BM (2/17, 11.8%) and normocellular BM (22/62, 35.5%) (p=0.002). Out of the 50 (50/83, 60.24%) patients who achieved CR, relapse occurred in 16% of the cases (8/50). Expressions of *HOXB4* and *PRDM16* were not significantly associated with CR or relapse. (Tables 3 and 4).

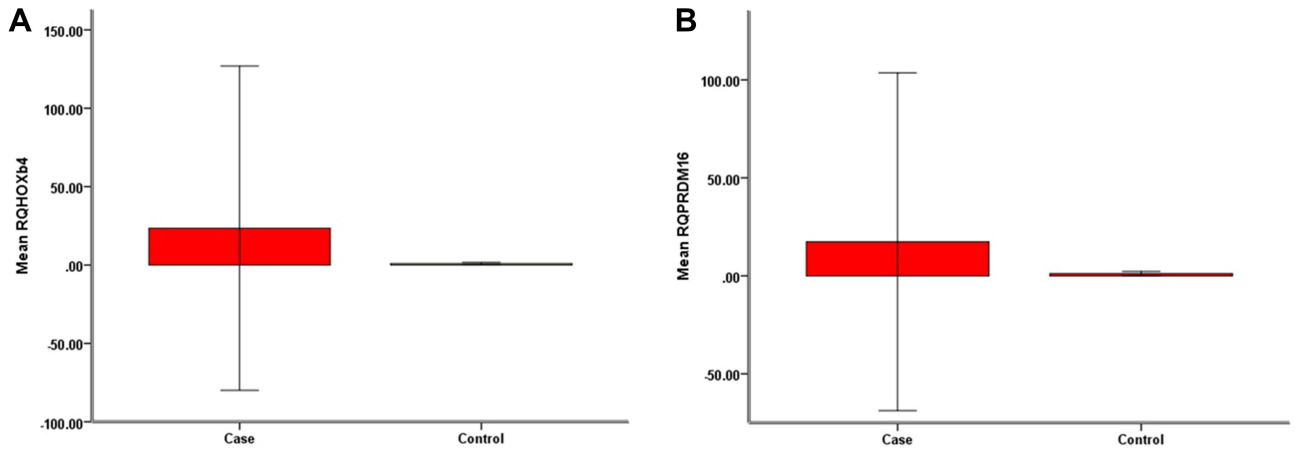

Figure 1 The mean fold change of *HOXB4* (**A**) and *PRDM16* (**B**) gene expressions.

Survival Analysis and Response to Treatment

The median follow-up period was 29.3 months. The survival analyses revealed no significant differences in the OS and DFS outcomes between *HOXB4* or *PRDM16* high and low expressers in AML patients (Table 5, Figure 2).

Table 2 Expression of *HOXB4* and *PRDM16*

Variables		AML	
		N=83	%
HOXB4	Low Expression	26	31.3
	High Expression	57	68.7
PRDM16	Low Expression	55	66.2
	High Expression	28	33.8

Table 3 Relation Between *HOXB4* and All Other Variables

Variables		*HOXB4*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Age: (years)*		38.50 (18–56)		40.00 (18–57)		−0.692	NS
TLC x10^9 /mm^3*		25.00 (1.3–358)		40.00 (0.50–616)		−1.164	NS
Hb (gm/dl)*		8.00 (4.0–12.0)		7.60 (3.7–13.2)		−0.692	NS
Platelets x10^9 /mm^3*		33.50 (10.0–225.0)		36.00 (5.0–826.0)		−0.191	NS
Peripheral blood blasts %*		38.50 (10.0–85.0)		47.00 (0.0–96.0)		−0.285	NS
BM blasts %*		49.50 (25.0–97.0)		63.00 (20–0–96.0)		−1.287	NS
Sex	Male	10	21.7	36	78.3	4.408	0.036 #
	Female	16	43.2	21	56.8		

(*Continued*)

Table 3 (Continued).

Variables		*HOXB4*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
BM cellularity	Normocellular	6	35.3	11	64.7	1.974	NS
	Hypercellular	20	32.3	42	67.7		
	Hypocellular	0	0.0	4	100.0		
FAB	M0	0	0.0	2	100.0	9.405	NS
	M1	3	25.0	9	75.0		
	M2	13	39.4	20	60.6		
	M3	2	100.0	0	0.0		
	M4	4	16.0	21	84.0		
	M5	3	50.0	3	50.0		
	M7	1	33.3	2	66.7		
IPT	Myeloid	18	36.7	31	63.3	4.479	NS
	Monocytic	3	50.0	3	50.0		
	Myelomonocytic	4	16.0	21	84.0		
	Megakaryoblastic	1	33.3	2	66.7		
Genetic risk	LR	13	41.9	18	58.1	4.268	NS
	IR	7	19.4	29	80.6		
	HR	6	37.5	10	62.5		
FLT3-ITD	Wild	22	28.5	55	71.5	0.012	NS
	Mutant	4	66.7	2	33.3		
NPM1 exon 12	Wild	7	24.1	22	75.2	2.419	NS
	Mutant	0	0.0	7	100.0		
CR	No	10	30.3	23	69.7	1.850	NS
	Yes	16	32.0	34	68.0		
Relapse	No	13	31.0	29	69.0	0.132	NS
	Yes	3	37.5	5	62.5		

Note: *Median (Min-Max), NS: non-significant, p value set significant at ≤0.05, # significant difference between high expression and low expression groups.

Discussion

AML is a malignant illness of the bone marrow defined by the arrest of hematopoietic precursors at an early stage of development. It is the most prevalent form of leukemia in adults and is associated with a poor prognosis.[30]

Additionally, genetic anomalies affect the progression and recurrence of AML, which may aid in targeting treatment and improving prognosis. AML is a clonal illness characterized by various genetic defects, but little is known about the molecular processes behind clinical variability within the same cytogenetic risk group.[31,32]

Table 4 Relation Between *PRDM16* and All Other Variables

Variables		*PRDM16*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Age: (years)*		39.00 (18–56)		45.50 (18–57)		−1.123	NS
TLC x10^9 /mm^3*		30.00 (0.5–616.0)		27.50 (1.90–242.0)		−0.356	NS
Hb (gm/dl)*		7.60 (3.8–13.2)		7.90 (3.7–12.0)		−1.243	NS
Platelets x10^9 /mm^3*		40.00 (5.0–283.0)		29.00 (9.0–826.0)		−1.146	NS
Peripheral blood blasts %*		42.00 (0.0–96.0)		43.50 (5.0–90.0)		−0.058	NS
BM blasts %*		57.00 (25.0–97.0)		64.50 (20.0–88.0)		−0.092	NS
Sex	Male	27	58.7	19	41.3	2.645	NS
	Female	28	75.7	9	24.3		
BM Cellularity	Normocellular	15	88.2	2	11.8	10.771	0.002 #
	Hypercellular	40	64.5	22	35.5		
	Hypocellular	0	0.0	4	100.0		
FAB	M0	1	50.0	1	50.0	8.276	NS
	M1	7	58.3	5	41.7		
	M2	26	78.8	7	21.2		
	M3	2	100.0	0	0.0		
	M4	12	48.0	13	52.0		
	M5	5	83.3	1	16.7		
	M7	2	66.7	1	33.3		
IPT	Myeloid	36	73.5	13	26.5	5.453	NS
	Monocytic	5	83.3	1	16.7		
	Myelomonocytic	12	48.0	13	52.0		
	Megakaryoblastic	2	66.7	1	33.3		
Genetic risk	LR	23	74.2	8	25.8	3.265	NS
	IR	20	55.6	16	44.4		
	HR	12	75.0	4	25.0		
FLT3-ITD	Wild	53	68.8	24	31.2	0.000	NS
	Mutant	2	33.3	4	66.7		
NPM1 exon 12	Wild	15	51.7	14	48.3	0.042	NS
	Mutant	5	71.4	2	28.6		
CR	No	19	57.6	14	42.4	0.132	NS
	Yes	36	72.0	14	28.0		

(*Continued*)

Table 4 (Continued).

Variables		*PRDM16*				Test	P-value
		Low Expression		High Expression			
		N	%	N	%		
Relapse	No	30	71.4	12	28.6	0.043	NS
	Yes	6	75.0	2	25.0		

Notes: *Median (Min-Max), p-value set significant at ≤0.05, # significant difference between high expression and low expression groups.
Abbreviation: NS, non-significant.

Table 5 Relation Between *HOXB4* and *PRDM16* Expressions and Survivals

Variables		OS					DFS				
		No=83	No. of Events	Median Survival Time	24-Month Survival Estimate	P-value	No=49	No. of Events	Median Survival Time	24-Month Survival Estimate	P-value
HOXB4	Low Expression	26	41	2.401	0.200	NS	16	3	NR	0.606	NS
	High Expression	57	18	5.395	0.280		33	5	26.908	0.646	
PRDM16	Low Expression	55	39	4.211	0.245	NS	36	6	26.91	0.660	NS
	High Expression	28	20	1.678	0.214		13	2	NR	0.700	

Note: *NR (median not reached).

The human *HOX* gene family consists of 39 members clustered on four distinct chromosomes.[33] Although overexpression of the *HOX* family has been seen in AML with normal karyotypes, the prognostic relevance of each *HOX* gene differs.[34] Additionally, *PRDM16* is a transcription factor required for the maintenance of hematopoietic stem cells. *PRDM16* has been documented to be mutated, translocated, or expressed abnormally in several subgroups of AML.[21,35–37]

We reported that the mean fold change of *HOXB4* and *PRDM16* expression was significantly higher in AML compared to the control. These results agree with Shiba et al,[23] who discovered overexpression of the *PRDM16* gene in 23% (84/369) of juvenile de novo AML patients after establishing an optimum *PRDM16* gene expression cutoff threshold. Further, Yamato et al[38] studied *PRDM16* expression in 151 AML patients and found that 47 (31%) individuals had elevated *PRDM16* expression. Our results are consistent with Umeda et al,[34] who examined the expression of newly defined hematopoietic stem cell factors including *HOXB4* in BM from de novo AML patients, and found that *HOXB4* was substantially more abundant in AML than in normal controls. In cell culture and murine BM transplantation assays, Bansal et al[39] reported that *HOX* genes also were dysregulated in leukemic BM with up-regulation of *HOXB4* mainly.

In addition, we reported that *FLT3-ITD* mutations were detected in 6 patients (7.2%), while *NPM1 exon 12* mutations were detected in 7 patients (19.4%) out of 36 patients with intermediate genetic risk. We found that all patients with *NPM1 exon 12* mutations and 33.3% of patients with *FLT3-ITD* mutations had high expression of *HOXB4*. On the other hand, our results revealed that 71.4% of patients with *NPM1 exon 12* mutations and 33.3% of patients with *FLT3-ITD* mutations had low expression of *PRDM16*. The relation between these mutations and the expressions of *HOXB4* and *PRDM16* may be illustrated by their impact on good CR and relapse rate.

Furthermore, we observed that the expression of *HOXB4* and *PRDM16* was not significantly associated with CR or relapse. Low expression of *HOXB4* and high expression of *PRDM16* were associated with CR of 32% and 28%, respectively. These results disagree with Yamato et al[38] and Umeda et al,[34] who observed that high *PRDM16* and low *HOXB4* expressions are significant predictive markers for poor prognosis in AML patients.

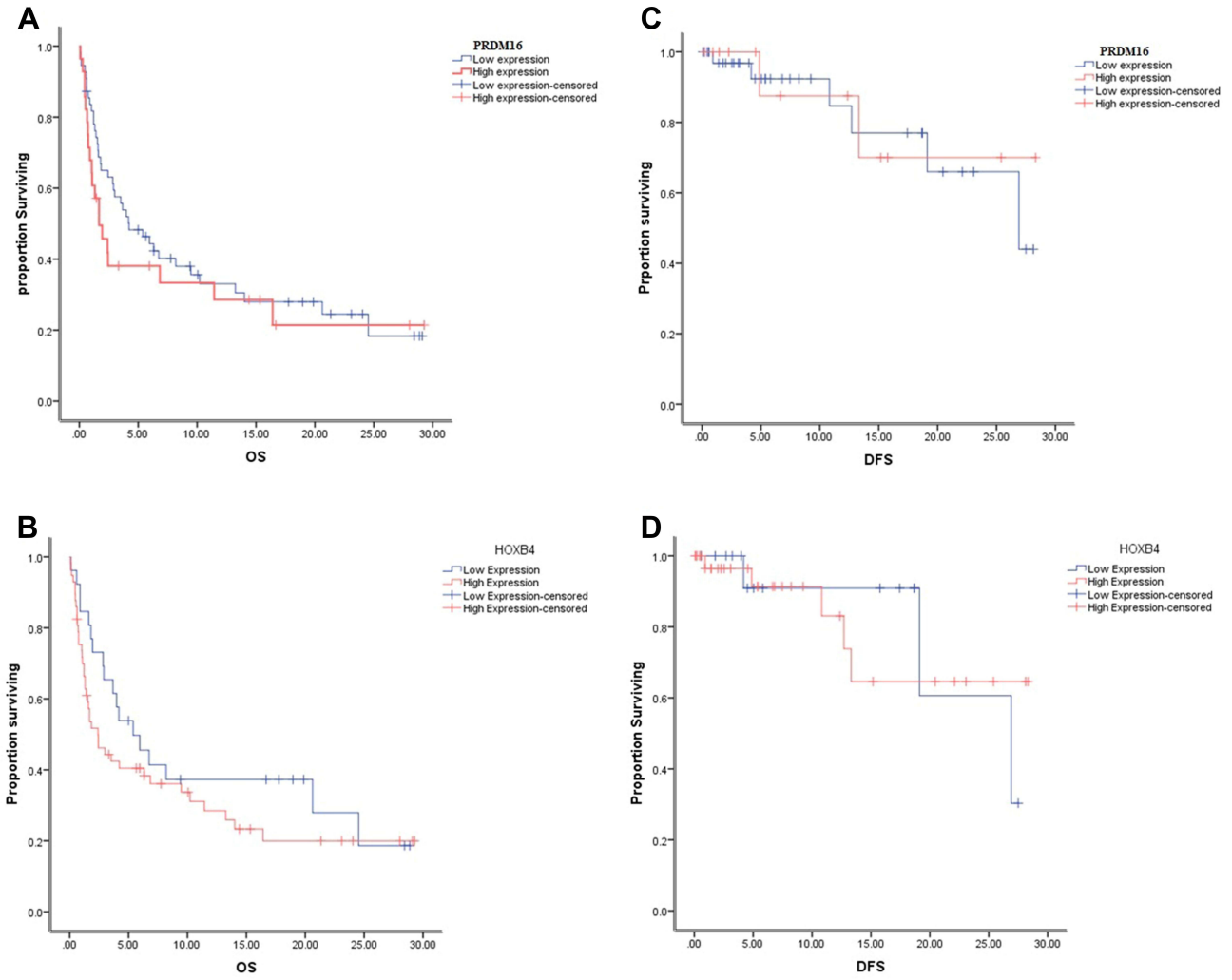

Figure 2 (**A** and **B**) Kaplan–Meier curves with log-rank statistics describe OS according to *PRDM16* and *HOXB4* expression level. (**C** and **D**) Kaplan–Meier curves with log-rank statistics describe DFS according to *PRDM16* and *HOXB4* expression levels.

Interestingly, concerning the OS, our analyses revealed a short OS and DFS in high *PRDM16* and low *HOXB4* expressions AML patients with no significant differences. In accordance with our finding, Shiba et al[23] reported that the OS among *PRDM16*-overexpressing patients was significantly worse than in patients with low *PRDM16* expression (51% vs 81%, $P < 0.001$). Further, the 5yr OS was significantly worse in high-*PRDM16*-expression patients than in low-*PRDM16*-expression patients (18% vs 34%; P=0.002), as reported by Yamato et al.[38]

Conclusion

High *PRDM16* and low *HOXB4* gene expressions may be used as poor prognostic and predictive markers in newly diagnosed AML adult patients, but larger studies are needed to prove these results. The correlation between *PRDM16* and *HOXB4* gene expressions and *FLT3-ITD* and *NPM1 exon 12* mutations might have a role in CR, relapse, OS, and DFS, however, this should be clarified in analysis with a larger number of samples.

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Disclosure

The authors declare no conflicts of interest in relation to this work.

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