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RESEARCH ARTICLE

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Dysregulation of miR-125b predicts poor response to therapy in pediatric acute lymphoblastic leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most well-known sort of leukemia in children. In spite of favorable survival rates, some patients relapse and achieve a poor outcome.

Methods: We analyzed miR-125b and Bcl-2 expressions in pediatric patients with ALL and evaluated their clinical utility as molecular markers for the prediction of disease outcomes.

Results: Downregulation of miR-125b and increased Bcl-2 expression levels in pediatric patients with ALL were associated with poor prognosis at diagnosis. At day 28 of induction, miR-125b was significantly increased, whereas Bcl-2 was downregulated. Loss of miR-125b during diagnosis and its elevation after therapy are strongly correlated with short leukemia-free survival and worse survival. Moreover, the combination of miR-125b with Bcl-2 markers can clearly enhance the prediction of the disease outcome. Finally, a univariate analysis highlighted the independent prognostic value of miR-125 in a pediatric patient with ALL.

Conclusions: miR-125b and Bcl-2 together are potent predictors for the prognosis and, therefore, can be used as therapeutic targets in childhood ALL.

KEYWORDS

Bcl-2, childhood acute lymphoblastic leukemia (ALL), miR-125b, prognostic factors

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most well-known sort of leukemia in children, accounting for approximately 25% of childhood malignancies.[1] In developed countries, the overall survival (OS) and leukemia-free survival (LFS) rates have improved in the last few years, approaching 80% and 90%, respectively.[2]

Throughout years, studies have established that epigenetic changes can prompt dysregulation of microRNAs (miRNAs) in leukemia, which in turn plays a critical role in the pathogenesis of hematological malignancies.[3] MicroRNA-125b is located on chromosomes 11q23 and is interpreted from two loci: has-miR-125b-1 and a-miR125b-2.[4] It has been demonstrated that miR-125b-1 is engaged with a few chromosomal translocations, such as t(2;21)(p21;q23) and t(11;14)(q24;q32), which mediate Myelodysplastic Syndrome (MDS)and Acute Myeloid Leukemia (AML) in addition to t(9; 22)(q34; q11) in B-cell acute lymphoid leukemia (B-ALL).[33] According to Sanddhya et al,[6] miR-125b overexpression was known to stimulate oncogenicity and shorten dormancy as a secondary occasion in leukemia. In addition, miR-125b is known to improve the proliferation of pre-B cells by the B cell–specific gene CD19 that is regulated epigenetically during early B cell improvement.[7,8] In this manner, miR-125b has a critical role in the differentiation of B-cell and can be utilized to approve therapeutic approaches like immunoconjugates and histone deacetylation inhibitors, which are known to regulate phenotypic variations of precursor B cells and aberrant miRNA regulation.[9]

Therefore, the precursor B cell markers CD10, CD19, and CD22 can play a critical role in miR-125b regulation.[6]

Bcl-2 proteins are key regulators of the intracellular apoptosis pathway and play an important role in cancer pathogenesis.[10] They promote cell survival in chronic lymphocytic leukemia (CLL),[11] cell invasion and metastases in colorectal cancer,[12] and hepatocellular carcinoma[13] and are associated with poor outcome in gallbladder cancer.[14] Bcl-2 is a direct target of miR-125b-5p and mediates its function.[14] Downregulation of miR-125b and miR-155 promotes proliferation of leukemic cells through suppression of Bcl-2.[15] Recent studies have focused on Bcl-2 as a therapeutic target in cancer. It was found that small molecule inhibitors have also been developed and are currently studied in clinical trials for CLL patients.[16-18] Although Bcl-2 inhibitors contribute to multiple drug resistance, miR-125b potentially overcomes drug resistance for cisplatin in gallbladder cancer[14] and osteosarcoma cell lines by targeting Bcl-2.[19] Yang et al[14] demonstrated that the miR-125b-5p/Bcl-2 pathway is a potential therapeutic target for gallbladder cancer.

Prompted by the involvement and the crucial role of miR-125b in hematological malignancies, the current study aims to criticize the expression profile of miR-125b and Bcl-2 in pediatric patients with ALL to evaluate their clinical significance as predictors for disease outcome and patients' response to chemotherapy.

2 | METHODS

The current study enrolled 120 children with de novo diagnosed ALL, admitted at the Ain Shams Children's Hospital, Cairo, Egypt between March 2015 and August 2017. Thirty children, not suffering from any hematologic or another type of malignancy, constituted our control cohort. The patients' clinical pathological characteristics are presented in Table 1. All patients provided their written informed consent in accordance with the Declaration of Helsinki. Peripheral blood (PB) samples were obtained with a minimum blast infiltration of 25%. All patients received a chemotherapy induction protocol. Matched PB samples after pathological evaluation were collected at day 28 after induction and included in the analysis. All sample handling, procedure, and storage were the same for leukemic and control subjects. Patients' follow up and stratification of risk groups of childhood ALL were done in accordance with the Berlin-Frankfurt-Munster (BFM) guidelines. The procedure included assessment of bone marrow (BM) blasts on day 8, BM blasts % on day 15, and minimal residual disease (MRD) measurement at day 28 of induction.[16] The diagnosis of ALL was done according to the morphologic assessment of the Leishman stained smears of the BM aspirates along with special immunocytochemical stains, immunophenotyping, and cytogenetic analysis. Assessment of MRD was performed using a lineage-specific monoclonal panel: for B-lineage ALL and T-lineage ALL. Brainstorm to BFM protocol guidelines. MRD was considered positive when leukemic cells exceeded 0.01% of all marrow nucleated cells on days 15 and 28.

2.1 | miRNA and messenger RNA extraction and purification

miRNA and total messenger RNA were extracted from mononuclear cells (MNCs) isolated by Ficoll-Hypaque density gradient centrifugation from 2 mL PB samples in ethylenediaminetetraacetic acid BM cells from newly diagnosed patients. Total RNA was extracted using a miRNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total RNA concentration and purity were evaluated spectrophotometrically at 260 and 280 nm. RNA integrity was visually confirmed by agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized by reverse transcription reaction using the miScript II RT kit (Qiagen).

2.2 | miRNA-125b expression analysis

The quantification of miR-125b levels was performed using the SYBR-green fluorescent-based primer assay

TABLE 1 Mean value of miR-125b and Bcl-2 expression levels among high/low risk prognostic features in pediatric patients with ALL

Variable	ALL N = 120	miR-125b expression cut-off value = 0.97			Bcl-2 expression cut-off value = 4.0		
		Low N, %	High N, %	*P* value	Low N, %	High N, %	*P* value
Age, y							
1-9	82 (68)	34 (79)	48 (62)	S	54 (67)	28 (33)	NS
<1, ≥10	38 (32)	9 (21)	29 (38)		28 (72)	10 (28)	
Sex							
Male	66 (55)	27 (63)	39 (51)	NS	47 (58)	19 (49)	NS
Female	54 (45)	16 (37)	38 (49)		34 (42)	20 (51)	
WBCs, cells/μL							
<50 000	94 (78)	33 (77)	61 (80)	NS	66 (82)	15 (18)	NS
≥50 000	26 (22)	10 (23)	16 (20)		28 (72)	11 (28)	
Hb, g/dL							
<10	99 (83)	30 (70)	69 (90)	HS	69 (85)	30 (77)	NS
≥10	21 (17)	13 (30)	8 (10)		12 (15)	9 (23)	
BM blasts, %							
<25%	98 (82)	26 (60)	72 (94)	HS	67 (83)	31 (80)	NS
≥25%	22 (18)	17 (40)	5 (6)		14 (17)	8 (20)	
Phenotype							
B-ALL	87 (73)	32 (74)	55 (71)	NS	58 (72)	29 (74)	NS
T-ALL	33 (27)	11 (26)	22 (29)		23 (28)	10 (26)	
Precursor B cells							
$CD10^+$	70 (80)	26 (81)	44 (80)	NS	47 (81)	23 (79)	NS
$CD10^-$	17 (20)	6 (19)	11 (20)		11 (19)	6 (21)	
High hyperdiploidy (>50 chromosomes)							
Yes	42 (42)	15 (40)	27 (44)	NS	29 (43)	13 (41)	NS
No	58 (58)	23 (60)	35 (56)		39 (57)	19 (59)	
Hypodiploidy (≤45 chromosomes)							
Yes	9 (45)	2 (40)	7 (47)	NS	5 (38)	4 (57)	NS
No	11 (55)	3 (60)	8 (53)		8 (67)	3 (43)	
Philadelphia chromosome BCR-ABL1/t(9;22)(q34;q11)							
Yes	47 (39)	18 (42)	29 (38)	NS	31 (38)	16 (41)	NS
No	73 (61)	25 (58)	48 (62)		50 (62)	23 (59)	
TEL-AML1/t(12;21)(p13;q22)							
Yes	34 (28)	10 (23)	24 (31)	NS	23 (28)	11 (28)	NS
No	86 (72)	33 (77)	53 (69)		58 (72)	28 (72)	
BFM risk groups							
Standard risk	79 (66)	17 (40)	62 (80)	HS	62 (76)	17 (44)	HS
Intermediate	20 (17)	14 (33)	6 (8)		12 (15)	8 (20)	
High risk	21 (17)	12 (28)	9 (12)		7 (9)	14 (40)	
BM blasts % at day 15							
M1 (blasts < 5%)	100 (83)	26 (60)	74 (96)	HS	69 (85)	31 (80)	NS
M2 (blasts 5–25%)	12 (10)	9 (21)	3 (4)		7 (9)	5 (12)	
M3 (blasts ≥ 25%)	8 (7)	8 (19)			5 (6)	3 (8)	
MRD on day 28							
<0.01%	78 (65)	16 (37)	62 (80)	HS	62 (76)	16 (41)	HS
>0.01%	17 (14)	15 (35)	2 (3)		10 (12)	7 (18)	
Unknown	25 (21)	12 (28)	13 (17)		9 (11)	16 (41)	
Treatment outcome							
CCR	90 (75)	27 (63)	63 (82)	HS	70 (86)	20 (51)	HS
Relapse	30 (25)	16 (37)	14 (18)		11 (14)	19 (49)	
Patients' survival							
Alive	97 (81)	30 (70)	67 (87)	S	74 (91)	23 (59)	HS
Dead	23 (19)	13 (30)	10 (13)		7 (9)	16 (41)	

Abbreviations: B-ALL, B lineage acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Munster; BM, bone marrow; CCR, complete clinical remission; CD, cluster of differentiation; MRD, minimal residual disease; T-ALL, T lineage ALL; WBCs, white blood cells. HS: Highly statistical significance; test is significant at <0.01 level, S: Statistical significant at ≤0.05.

(has-miR-125b-5p; cat no: MIMAT0000423) and the small nucleolar RNA, C/D box 48 (SNORD48), (NCBI RefSeq: NR_002745.1) as a reference gene. The specific forward (hybridized to miR-125b- or SNORD48-specific sequences of cDNA template) and universal reverse (hybridized to poly [T] adapter reverse transcription primer sequence of cDNA template) primer sequences were investigated using the miScript SYBR green PCR kit (Qiagen). The quantitative polymerase chain reaction (qPCR) was performed in the 5-plex Rotor-Gene PCR system (Qiagen). The 20 μL reaction mixture/reaction consist of 2× QuantiTect cyber green PCR master mix, 10× miScript universal primer, 2 μL primer assay and 50 pg to 3 ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consisted of 15 minutes of HotStarTaq DNA polymerase activation at 95°C followed by 40 cycles of denaturation at 94°C for 15 minutes, primer annealing for 30 seconds at 55°C, and extension at 70°C for 30 seconds). The fluorescence data were collected at the extension step. After amplification, melting curve analysis of the products was performed from 60 to 95°C at 0.3°C temperature increment intervals for 30 seconds. The melting temperature (T_m) of the products was determined by plotting the negative first derivative of the normalized fluorescence vs temperature. Only samples with a single peak at the appropriate T_m (temperature of the peak maximum) for both miR-125b and SNORD48 were included in the analysis of this study. The $2^{\Delta\Delta C_t}$ method was used for the analysis of miR-125b expression levels, using SNORD48 as an endogenous reference control for normalization purposes. The amplification efficiencies of the target miR-125b-5p and the reference SNORD48 genes were assessed by a validation experiment, using serial dilutions of a control cDNA covering six orders of magnitude (1–10_5 ng cDNA) as a template. The linear increases of miR-125b and SNORD48 calibration curves highlight the 106.3% and 104.2% amplification efficiencies, respectively, as well as the absence of PCR inhibition by the template.

2.3 | Bcl-2 expression analysis

For the detection of the Bcl-2 expression, cDNA was amplified using a Hs_Bcl-2_1_SG QuantiTect primer assay cat no: QT00025011 and Hs_ACTB_1_SG QuantiTect primer assay (NM_001101) was used as the reference gene. Each sample was amplified by both primers using QuantiTect SYBR green master mix (Qiagen). The 25 μL reaction mixture/reaction consisted of 2× QuantiTect cyber green PCR master mix, 0.3 μM primer assay, and 500 ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consisted of 15 minutes of HotStar-Taq DNA polymerase activation at 95°C followed by 45 cycles of denaturation at 94°C for 15 minutes, primer annealing for 30 seconds at 52°C and extension at 72°C for 30 seconds. The fluorescence data were collected at the extension step. After amplification, the Bcl-2 expression was calculated using the $2^{\Delta\Delta C_t}$ method. Test validation and amplicon specification were confirmed by the same methods used in miRNA-125b.

2.4 | Statistical Analysis

Statistical analysis was performed using the SPSS v.22 (IBM, Chicago, IL). The nonparametric Mann-Whitney U test and the Wilcoxon Signed Rank test were performed to evaluate the differences in the miR-125b expression between leukemic and healthy specimens, and between samples on diagnosis and on the day 28 of the induction protocol, respectively. Moreover, to assess the correlation of miR-125b levels and day 33/diagnosis miR-125b expression levels ratio with patients' clinic-pathological features, the receiver operating curve characteristics analysis (ROC) was performed to assess the discriminatory ability of miR-125b for childhood ALL. Spearman's correlation was used to assess whether the expression levels of miR-125b and Bcl-2 were correlated with the patient outcome. OS was calculated from diagnosis to death or last follow-up and LFS from complete remission (CR) to relapse or death. Both OS and LFS were estimated with the Kaplan-Meier method and comparisons among subgroups of patients with high and low values of miR-125b and Bcl-2 genes expressions at diagnosis/day 28 after induction were performed using the logrank test. Significance was set at a value of less than or equal to 0.05.

3 | RESULTS

3.1 | Expression of miR-125b and Bcl-2 in pediatric patients with ALL

Pediatric ALL BM samples exhibit a lower expression of miR-125b ($P < 0.001$) compared with healthy controls. In contrast, the Bcl-2 expression levels were dramatically higher in ALL vs controls ($P < 0.001$) (Supporting Information Table S1; Figure 1). Spearman's correlation was conducted to find out the correlation between miR-125b and Bcl-2 expression levels in pediatric patients with ALL. An insignificant negative correlation was found: the low value of miR-125b at diagnosis was associated with high values of Bcl-2, P value greater than 0.05. Receiving operator characteristic (ROC) curve analysis illustrated that miR-125b and Bcl-2 expressions were potential biomarkers for screening pediatric patients with ALL from healthy controls with an area under the curve of 0.99, 0.9 reciprocally. However, miR-125b was superior to Bcl-2 in test sensitivity and specificity; at a cut-off less than or equal

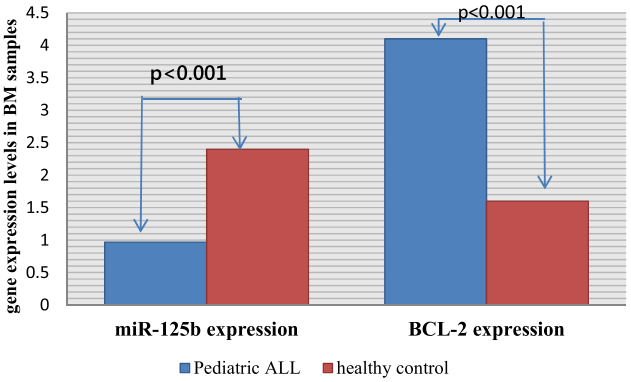

FIGURE 1 Downregulation of miR-125b expression and overexpression of Bcl-2 in pediatric acute lymphoblastic leukemia (ALL) patients at diagnosis compared with healthy controls, miR-125b expression in the BM of pediatric patients with ALL patients were significantly decreased (ALL at diagnosis vs normal: 0.97 ± 0.34 vs 2.4 ± 0.8; $P = 0.001$); Bcl-2 expression level in pediatric patients with ALL was dramatically higher than that in healthy controls (ALL at diagnosis vs normal [median (IQR): 4.1 (2.8-5.5) vs 1.56(1.2-2.6); $P = 0.001$]). BM, bone marrow

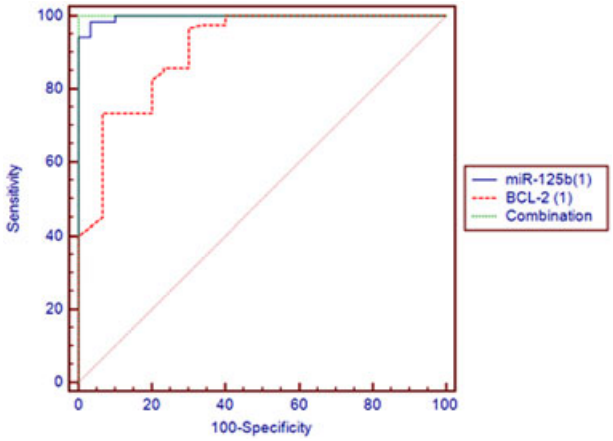

FIGURE 2 ROC curve analysis illustrated that the BM miR-125b and Bcl-2 levels were potential biomarkers for screening pediatric patients with ALL from healthy controls with the area under the ROC curve (AUC) of 0.99 and 0.9, cut-off ≤1.5 and >2.14 respectively. Based on this cut-off value for each, the sensitivity and specificity of miR-125b and Bcl-2 level for distinguishing ALL from healthy controls were 98.3% and 96.7%; 96.6% and 70% respectively. Combination of both shows 100% sensitivity and specificity. ALL, acute lymphoblastic leukemia; BM, bone marrow; ROC, receiver operating curve

to 1.5, the miR-125b sensitivity and specificity were 98% and 96.7% respectively vs 96.7% and 70% for Bcl-2 at a cut-off value of greater than 2.14 (Figure 2; Supporting Information Table S2).

3.2 | Lower miR-125b/higher Bcl-2 expressions were associated with high risk ALL group

miR-125b presented a wide variability of expression of ALL patients at diagnosis (Supporting Information Table S3). It was found that lower miR-125b and higher Bcl-2 expression levels at diagnosis were correlated with unfavorable prognostic features of childhood ALL (Supporting information Tables S3 and S4). Literally, reduced miR-125b and high Bcl-2 levels were detected in the high-risk age group ($P < 0.05$), in high BM blast count at diagnosis, and at day 15 after induction ($P < 0.001$) as well as in patients with a low hemoglobin concentration ($P < 0.001$; Supporting Information Tables S3 and S4).

3.3 | The relation between miR-125b and Bcl-2 expression and treatment response parameters

Even though a low sample size was studied in each ALL subgroup, the expression of miR-125b was lower and Bcl-2 was higher in patients who presented BM status at intermediate/high risk at day 28 of induction ($P < 0.001$). In addition, a similar result was obtained regarding MRD (>0.01 vs >0.01) and clinical response to therapy when miR-125b and Bcl-2 expression levels were compared in ALL patients who achieved CR and those who relapsed ($P < 0.001$; Supporting Information Tables S3 and S4).

3.4 | The expression of miR-125b is significantly reduced in childhood ALL on diagnosis and increases at day 28 after induction

The evaluation of miR-125b expression levels from PB MNCs specimens obtained on day 28 after induction indicated that miR-125b is significantly increased in pediatric patients with ALL at the end of the induction protocol ($P < 0.001$). On the other hand, the Bcl-2 expression levels were dramatically decreased at day 28 after induction ($P < 0.001$)). Finally, miR-125b showed significantly increased, 1.7 fold, and decreased 13.6 fold Bcl-2 levels compared with the primary sample at diagnosis ($P < 0.001$; Supporting Information Table S5; Figure 3). These results support the hypothesis that the upregulation of miR-125b levels and downregulation of Bcl-2 on day 28 after induction reflects patients' good response to treatment.

3.5 | miR-125b/Bcl2 associated pathway is a predictor biomarker for disease progression and patients' survival outcome in pediatric ALL

To appraise if the miR-125b and Bcl-2 gene expressions are significant prognostic biomarkers in pediatric patients with

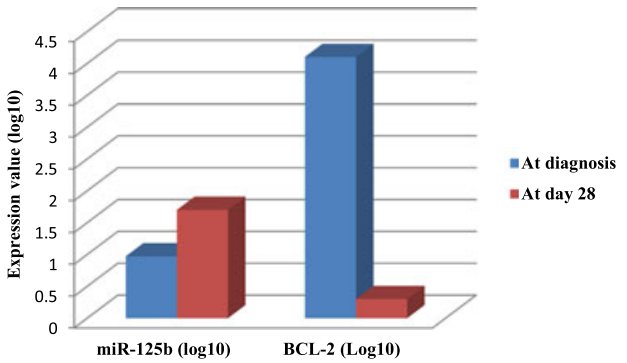

FIGURE 3 Bar graph representing miR-125b and Bcl-2 expression levels ratio in childhood ALL on disease diagnosis and on day 28 after induction

ALL, logrank test and Kaplan-Meier curves were used for OS and LFS stratified according to miR-125b and Bcl-2 expression levels in pediatric ALL (Supporting Information Tables S6 and S7; Figure 4). We found that the pediatric patients with ALL who expressed miR-125b at a low level either at diagnosis or at day 28 of therapy had shorter LFS and OS ($P < 0.001$; Supporting Information Table S8; Figure 5). In addition, univariate analysis showed that the OS and LFS in the patients with low hemoglobin (g/dL; $P < 0.05$), BM blasts count at diagnosis as well as at day 15 of therapy, MRD > 0.01, BFM BM status, low miR-125b and overexpression of Bcl-2 ($P < 0.001$) (Tables were all significantly shorter than those of the low-risk group. Certainly, the multivariate Cox analysis was performed to evaluate the independent prognostic value of miR-125b for childhood ALL. The multivariate Cox models were adjusted for hemoglobin concentration, BM blast % at diagnosis, BM response on day 15, risk group according to BFM stratification and MRD, miR-125b and Bcl-2 expression values at disease diagnosis. Multivariate analysis (Tables 2,3) highlighted that all these parameters have no impact on OS and LFS in childhood ALL.

4 | DISCUSSION

It has been known that miR-125b promotes apoptosis by negatively regulating Bcl-2 in leukemia. Considering the

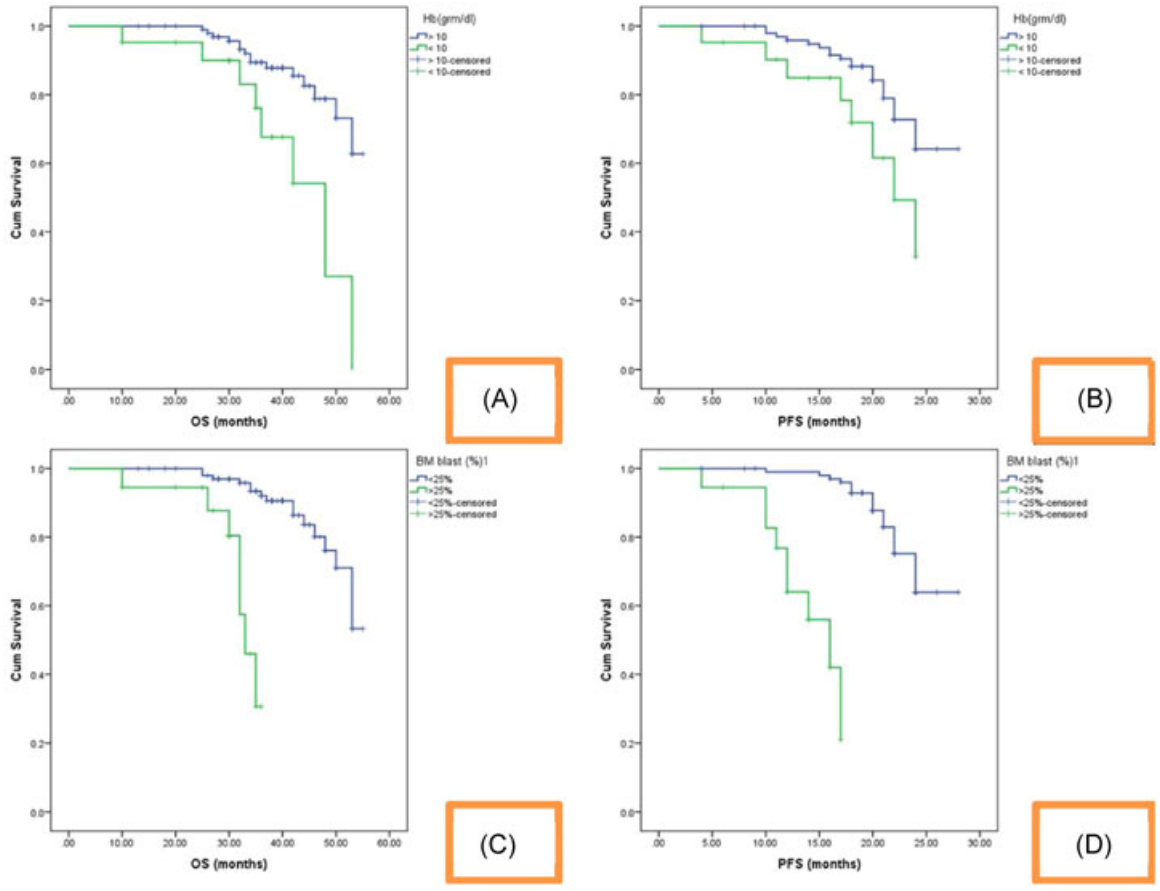

FIGURE 4 Downregulation of miR-125b levels on disease diagnosis in BM samples of pediatric patients with ALL is associated with poor outcome. Kaplan-Meier survival curves for the OS and PFS according to miR-125 levels on disease diagnosis for (A,B) the favorable and unfavorable Hb (g/dL), for (C,D) BM blasts % ALL patients' cohort. *P* values calculated by logrank test. ALL, acute lymphoblastic leukemia; BM, bone marrow; Hb, hemoglobin; OS, overall survival; PFS, progression free survival

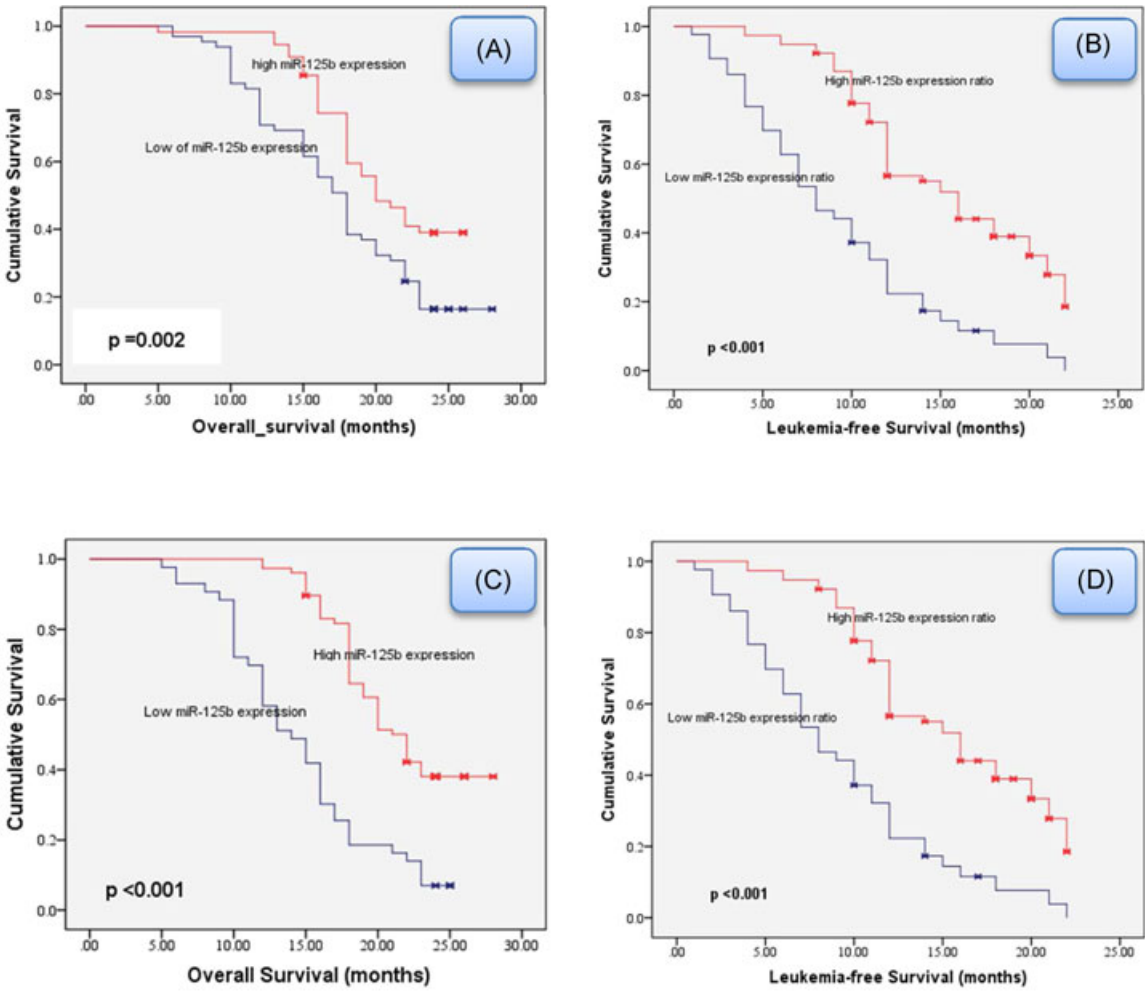

FIGURE 5 Downregulation of miR-125b levels on disease diagnosis and upregulation of miR-125b on day 33 are associated with poor prognosis. Kaplan-Meier survival curves for the OS and LFS according to miR-125 levels on disease diagnosis, and miR-125b levels at day 28/diagnosis for (A,B) and (C,D) respectively. The total childhood ALL patients' cohort, and *P* values calculated by logrank (Mantel-Cox) test. ALL, acute lymphoblastic leukemia; LFS, leukemia-free survival; OS, overall survival

fact that Bcl-2 is expressed in many neoplasms and involved in drug resistance, accordingly, miR-125b may be a new therapeutic agent in the future.[19,20]

Over the past few decades, there has been a good progress in pediatric ALL treatment protocols; thus, remission rates and survival expectancy start to increase.[21,22] Studies have highlighted the involvement of miRNAs pathways in leukemogenesis, aimed at improving disease morbidity and mortality.[23] The current study has demonstrated the prognostic value of miR-125b and Bcl-2 and its host gene in a series of pediatric patients with ALL. The expression of miR-125b was downregulated, and Bcl-2 was upregulated in pediatric patients with newly diagnosed ALL compared with normal healthy controls. ROC analysis and regression analysis verified that miR-125b and Bcl-2 could efficiently distinguish leukemic from normal cells either as an individual or combined biomarkers. Furthermore, patient's follow-up samples at 28th day after induction revealed a significant overexpression of miR-125b and reduction of Bcl-2 on day 28 of BFM treatment protocol. In addition, the low miR-125b expression ratio obtained from its low expression level at disease diagnosis and the high expression on day 28/diagnosis were correlated with unfavorable prognostic features, such as high blasts cell percentage, higher BFM-risk stratification, and increase in the age of diagnosis.

miR-125b has a controversial role in the pathogenesis of cancer: it either acts as an oncogene or as a tumor-suppressor gene depending on the cell context.[24] Our results are consistent with Schotte et al,[25] who observed a downregulation of miR-125b in leukemic mononuclear BM or PB cells in pediatric patients with ALL at diagnosis. These patients were negative to BCR-ABL, E2A-PBX, TEL-AML1, and hyperdiploidy compared with normal

TABLE 2 Univariate and multivariate Cox regression analysis for factors affecting OS (months)

	Univariate				Multivariate			
			95.0% CI for OR				95.0% CI for OR	
Compared groups	*P* value	Odds ratio (OR)	Lower	Upper	*P* value	Odds ratio (OR)	Lower	Upper
Hb, g/dL, (≥10 vs <10)	0.004	3.572	1.504	8.49	0.663	1.238	0.475	3.228
BM blast % at diagnosis (>25% vs <25%)	0.000	12.052	4.018	36.14	0.087	8.363	0.737	94.85
BM blasts % at day 15 (M3 vs M1,M2)	0.000	3.199	1.720	5.951	0.315	2.126	0.488	9.258
MRD at day 15 (>0.01 vs <0.01)	0.000	4.565	2.480	8.40	0.091	6.978	0.733	66.43
BFM (high vs low, intermediate)	0.000	7.541	3.547	16.03	0.068	2.748	0.928	8.14
miR125b (log^{10}) at diagnosis (low vs high)	0.000	0.07	0.022	0.23	0.907	1.099	0.227	5.32
Bcl-2 (log^{10}) at diagnosis (high vs low)	0.000	1.292	1.157	1.44	0.258	1.079	0.946	1.232

Abbreviations: BFM, Berlin-Frankfurt-Munster; BM, bone marrow; CI, confident interval; Hb, hemoglobin; MRD, minimal residual disease.

CD34 cells, as well as in MLL-rearranged patients. Similar results supporting the tumor suppression role of miR-125b have been reported by Tili et al;[26] they reported the reduced expression of miR-125b in aggressive and indolent CLL patients and explained its role in the metabolic adaptation of the cells to a transformed state via targeting key enzymes such as PCTP, SCD1, AKT2, and PDK1. In addition, miR-125b has a critical role in regulating cell apoptosis. It was declared by Busch et al[27] that miR-125b regulates p53 activity as an inhibitor in myeloid cells by targeting the 5-lipoxygenase metabolic enzyme and promotes BCR-ABL fusion protein tumorigenicity. Li et al[28] observed that downregulation of miR-125b is directly affected by the expression of BAK1, thus leading to upregulation of proapoptotic factors and downregulation of antiapoptotic factors in the mitochondrial apoptosis pathway.

The increased expression level of miR-125b has been reported in many cancers, including prostate cancer,[29] breast cancer,[30] head and neck cancer,[31] and leukeamia,[32] which suggested that miR-125b influences early hematopoiesis; thus its dysregulation can transform cells and induce leukemia, which affects both myeloid and lymphoid lineages.[52,53] Numerous studies have focused

TABLE 3 Univariate and multivariate Cox regression analysis for factors affecting PFS (months)

	Univariate				Multivariate			
			95.0% CI for OR				95.0% CI for OR	
Compared groups	*P* value	Odds ratio (OR)	Lower	Upper	*P* value	Odds ratio (OR)	Lower	Upper
Hb, g/dL, (≥10 vs <10)	0.036	2.381	1.057	5.366	0.300	0.627	0.259	1.516
BM blast % at diagnosis (>25% vs <25%)	0.000	31.872	9.057	112.160	0.006	21.057	2.384	185.94
BM blasts % at day 15 (M3 vs M1,M2)	0.000	4.516	2.580	7.903	0.132	2.233	0.786	6.346
MRD at day 15 (>0.01 vs <0.01)	0.000	4.031	2.478	6.557	0.011	4.765	1.431	15.86
BFM (high vs low, intermediate)	0.000	5.867	3.419	10.070	0.127	1.991	0.823	4.817
miR125b (log^{10}) at diagnosis (low vs high)	0.000	0.106	0.040	0.278	0.255	2.169	0.572	8.218
Bcl-2 (log^{10}) at diagnosis (high vs low)	0.000	1.247	1.131	1.374	0.305	1.064	0.945	1.198

Abbreviations: BFM, Berlin-Frankfurt-Munster; BM, bone marrow; CI, confident interval; Hb, hemoglobin; MRD, minimal residual disease; PFS, progression-free survival.

on the subsets of pediatric patients with ALL with specific chromosomal abnormalities; they noticed overexpression of miR-125b in progenitor B-ALL patients who were carrying the translocation (11;14)(q24; q32),[5,33,34] and also in patients positive for TEL-AML1.[9,35]

The low expression of miR-125b in BM blasts was dramatically correlated with the aggressiveness of pediatric patients with ALL. Univariate analysis demonstrated that the miR-125b level was an independent of other prognostic factors, including the cytogenetic abnormalities, phenotype, and initial WBC count. These might be explained by underestimation because our studied cohort was small. Moreover, the prognostic value of miR-125b was significantly associated with the BFM risk subgroups ($P < 0.001$), BM blasts %, and hemoglobin concentration. These findings highlight the prognostic potentials of miR-125b in pediatric patients with ALL. The clinical utility of miR-125b as a prognostic marker in childhood ALL and its relation to clinic-pathological characteristics was evaluated by[36] who established that miR-125b has a prognostic significance in BFM-treated patients with precursor B-ALL and high BM blast % at disease diagnosis, thus confirming the blasts mediated expression of BM miR-125b levels.

Focusing on patient's outcome and its correlation to miR-125b/Bcl-2 expression levels, downregulation of miR-125b associated with upregulation of Bcl-2 in pediatric patients with ALL at diagnosis, which was significantly associated with a high risk of relapse and a poor OS of the treated patients. In addition, increased expression of miR-125b is associated with Bcl-2 down regulation after induction predicts good patient's outcome, which reflects a good response to therapy and further confirms the regulatory role of miR-125b on Bcl-2. Similar results were reported by[14] and his colleagues, who demonstrated that downregulation of miR-125b increases the sensitivity to cisplatin in treated gallbladder cancer cells and mouse models through the direct suppression of Bcl-2. In addition to[36] who studied the clinical utility of BM miR-125b expression levels for the prognosis of BFM treatment outcome in childhood ALL, he reported that decreased expression of miR-125b at diagnosis and its overexpression on day 33 of BFM therapy were strongly associated with short survival and predicted poor response to BFM treatment. It can predict the value of MRD on day 15 and the current BFM-risk stratification system. The same expression pattern of miR-125b can ameliorate despite the presence of favorable WBC greater than 50 000 cells/mL, good prednisone response. Previous studies correlate miR-125b expression levels with chemotherapy resistance, upregulation of miR-125b mediates resistance to vincristine and childhood acute megakaryoblastic leukemia cells[37-39], and childhood precursor B-ALL patients resistant to vincristine and daunorubicin.[40] Moreover, it promotes survival against apoptotic stimuli and vincristine resistance in ETV6/RUNX1 leukemic cells.[35,39] In addition, miR-125b downregulation contributes to resistance for daunorubicin in leukemic cell lines.[41,42]

The prognostic impact of miR-125b was enriched with simultaneous determination of Bcl-2, which allowed us to build a simple score based on these two factors. We have observed in the current study that low expression of miR-125b and high Bcl-2 expression are independent prognostic factors associated with poor outcome in pediatric patients with ALL. Several studies clearly demonstrate the possibility of miR-125b in inducing apoptosis and inhibiting the activity of chemotherapeutic drugs, thus supporting our findings concerning the poor outcome.[36] Focusing on apoptosis-related targets, it was found that overexpression of miR-125b inhibits apoptosis in acute promyelocytic leukemia by targeting BAK1 proapoptotic gene,[41] Bcl-2 in ovarian,[43] and breast cancer.[44] Other networks with several genes involved in apoptotic pathways were observed by different studies as validated targets of miR-125b, such as TP53,[45,46] TP53INP1,[47,48] TNFAIP3,[46,49] and MAPK14 (p38a).[50] It was found that ectopic expression of miR-125b block cell apoptosis through suppression of p53 or BAK1 pathways[51] and NF-KB activation.[49]

In conclusion, our data support the convincing evidence that miR-125b expression may be markedly and consistently decreased in pediatric patients with ALL and in turn contributes to poor disease outcome and malignancy progression. miR-125b may serve as a potential prognostic marker for pediatric patients with ALL. In addition, it is a good monitor for the response to treatment. Kaplan-Meier survival analysis apparently distinguished the high risk for short-term relapse and worse OS from those who achieved a better outcome; patients who reveal downregulated miR-125b at diagnosis present poor disease outcome and bad response to therapy in pediatric patients with ALL.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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