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ORIGINAL RESEARCH

Proviral Integration of Moloney Virus-2 (PIM-2) Expression Level as a Prognostic Marker in Patients with Acute Myeloid Leukemia

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Purpose: This study aimed to assess *PIM-2* gene expression level as a prognostic marker in AML patients and to correlate the results with their clinical outcome.

Patients and Methods: This study was conducted on 50 de novo younger AML patients (median age 44). Quantitative real-time polymerase chain reaction (QRT-PCR) was used to assess the expression level of the *PIM-2* gene. The transcription level of the target gene (*PIM-2*) was normalized to that of the reference gene (GAPDH). Twenty control samples were withdrawn from 20 age- and sex-matched individuals for the analysis of the results using the $2^{-\Delta\Delta CT}$ method. On day 28 following induction chemotherapy, patients' bone marrow (BM) was examined for evaluation of their remission status.

Results: *PIM-2* gene expression was higher among AML patients who did not achieve complete remission (CR); also, it was higher in patients in the intermediate and poor cytogenetic risk groups. A significant positive correlation was found between PIM-2 level and BM blasts on day 28. In AML patients, PIM-2 has been discovered to be an independent predictive factor for achieving CR following standard induction treatment. Receiver operating characteristic curve (ROC) and area under the curve (AUC) were performed for PIM-2 level at diagnosis to evaluate its role in achieving remission after induction. It was found that PIM-2 at cutoff ≤1.6 had an AUC (0.903) with a sensitivity (90.48%) and specificity (86.21%), P <0.001.

Conclusion: Overexpression of the PIM-2 gene is associated with induction failure and low CR.

Keywords: AML, *PIM-2* gene, QRT-PCR, induction chemotherapy, complete remission

Introduction

Acute myeloid leukemia (AML) is the most aggressive myeloid neoplasm triggered by the acquisition of genetic alterations that lead to the transformation of hematopoietic progenitor cells.[1,2] In Egypt, leukemia comprises 10% of all malignancies, with AML representing 16.9% of the cases.[3] Despite many AML patients responding to induction chemotherapy, the refractory disease is frequent, and relapse represents the principal cause of treatment failure, so identifying the precise clinical outcome indicators can aid in developing a tailored treatment for particular patients.[4] One of the most important phenomena that leads to malignant cells' accumulation in leukemia patients is apoptosis inhibition, and such finding guided the researchers to target the anti-apoptotic factor, Proviral Integration of Moloney virus-2 (PIM-2) which belongs to a family of serine/threonine kinase (PIM-1, PIM-2 and PIM-3).[5]

Several human cancers, particularly hematological malignancies, showed an increased expression of PIM kinases, where they aid the survival and proliferation of malignant cells.[6] PIM kinases were found to be downstream effectors of vital oncoproteins like Janus Kinase 2 (JAK2), FMS-like tyrosine kinase 3 (FLT3) and Abelson (ABL)[7]; therefore, the PIM kinases have emerged as prospective pharmacological targets in a variety of hematological cancers with numerous Inhibitors demonstrating their efficacy in AML preclinical models.[8] SGI-1776, a pan-PIM inhibitor has been shown to be effective in AML cell lines for inhibiting PIM-2 kinase and inducing apoptosis.[9] PIM-2 plays a critical role in the

survival, proliferation and differentiation of hematopoietic cells as many cytokines implicated in the growth and maturation of these cells (especially IL3) regulate the expression of the PIM-2 gene at both protein and mRNA levels.[10] Over-expression of PIM-2 has been linked to the advancement of B-lymphoid malignancies as chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) and mantle cell lymphoma (MCL).[11,12] Almost half of DLBCL cases revealed elevated PIM kinase expression, especially in the activated B-cell (ABC) subtype, because of constitutive activation of JAK/STAT3 signalling.[13] A strong link between PIM kinase expression, STAT signaling, increased rates of proliferation, and progressive disease stages has been established.[14]

Several reports have revealed that PIM-2 is closely linked to myeloma cell growth and survival, so that it might be a promising therapeutic target.[15] PIM-2 has also been reported to be persistently expressed in primary AML blasts and to have a role in protein translation through regulating 4E-BP1 phosphorylation.[16] Elevated *PIM-2* gene expression level was found to be linked to a low rate of complete remission (CR) in AML patients and their poor survival, suggesting another aspect of its possible prognostic significance.[17] This study aimed to assess PIM-2 expression level in AML patients, correlate between its level, clinical and laboratory findings, response to treatment and patients' outcome.

Materials and Methods

The institutional review board (IRB) of Zagazig University approved the study (approval number, ZU-IRB# 4240), and written informed consent was taken from all patients involved in the study. The study was conducted according to the ethical principles of the declaration of Helsinki. The study was carried out at Clinical Pathology Department, Zagazig University Hospitals. Fifty de novo AML patients admitted to Medical Oncology Department, Clinical Hematology Unit, Internal Medicine Department, Zagazig University Hospitals were included in this study between August 2019 and September 2020. Following informed permission and IRB approval, peripheral blood (PB) and bone marrow (BM) samples were taken when the diagnosis was made. Patients' inclusion criteria include approval to participate in the study, newly diagnosed AML patients before receiving induction chemotherapy with no concurrent malignancy. Therapy-related AML and patients diagnosed with Acute Promyelocytic Leukemia (M3) were excluded. The patients were subjected to the following: Thorough history taking, clinical examination, routine lab investigations including Complete Blood Count (CBC) using Sysmex Xn (Sysmex, Japan), liver, kidney function tests and serum lactate dehydrogenase (LDH) using Cobas 6000 autoanalyzer (Roche Diagnostics, Germany), prothrombin time (PT) and activated partial thromboplastin time (APTT) using CS-1500 (Sysmex, Japan), erythrocyte sedimentation rate (ESR) using VISION-B (YHLO Biotech, China), BM aspiration and evaluation using Leishman and peroxidase stains, flow cytometry was used to determine immunophenotyping using FACSCanto II flow cytometer equipped with Diva software (Becton Dickinson, USA) and cytogenetic analysis (karyotyping) was performed with an image analyzer (Imstar, France) utilizing the G banding technique. Patients were categorized according to their cytogenetic risk stratification into favorable cytogenetic risk group (n=9 patients), Intermediate cytogenetic risk group (n=39 patients); 36 of them were with normal karyotyping, and the adverse cytogenetic risk group (n=2 patients). Radiological investigations such as Pavli-abdominal sonography, echocardiography and chest X-ray were done for the patients.

PIM-2 Quantitative Polymerase Chain Reaction (q PCR)

RNA was extracted from whole blood by RNA extraction kit (Gene JET RNA Purification Kit Catalog No. K0731 Thermo Scientific, USA). Its purity was determined using a Nanodrop2000 spectrophotometer (Thermo Scientific, USA). RNA was reverse transcribed to generate cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Part Number: 4375575) through Veriti thermal cycler (Applied Biosystems, Japan). TaqMan gene expression assay (Thermo Fisher) was used to perform quantitative PCR with primers and hydrolysis probes for PIM2 (ref. Hs00179139_m1) and an internal control GAPDH (ref. Hs03929097_g1) (Applied Biosystems). The transcription level of the target gene (*PIM-2*) was normalized to that of the reference gene (GAPDH). Control samples were withdrawn from 20 age- and sex-matched individuals. They were selected from patients with hematological diseases to whom BM examination was one of the required investigations (hypersplenism and idiopathic thrombocytopenic purpura). Relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method.[18]

Treatment Plan and Follow-Up

Patients were treated by an induction regimen (7+3) that included a 7-day cytarabine infusion ($100mg/m^2$) and 3 days of doxorubicin ($25mg/m^2$) treatment. Low-intensity treatment: lower dose chemotherapy or hypomethylation agents were used to treat patients over 60 years old.[19] Follow-up of the patients was done on day 28 after receiving induction chemotherapy by performing CBC and BM aspirate to evaluate their remission status. CR was defined as normocellular BM containing less than 5% blast cells and peripheral blood with at least 1×10^9/L neutrophils and $\geq100\times10^9$/L platelets.[20] Patients who achieved remission received three to four courses of high-dose cytarabine ($1–2$ g/ m^2 every 12 h on days 1, 3 and 5; total, 12 g/ m^2) as a consolidation.[19]

Statistical Analysis

The collected data were computerized and statistically analyzed using Statistical Package for Social Science program (SPSS version 24) (IBM; Armonk, New York, USA). The Shapiro–Wilk test was used to determine if the data had a normal distribution. Frequencies or relative percentages were used to express qualitative data. The difference between qualitative variables was calculated using the Chi-square test ($\chi2$) and Fisher exact test. The median and range were used to express quantitative data. Mann–Whitney test was used to calculate the difference between quantitative variables in two groups. Kruskal–Wallis Test was used to calculate the difference between quantitative variables in more than two groups. Spearman correlation tests were used for correlating variables, respectively. Univariate and multivariate logistic regression analyses were performed with P<0.1 in univariate analysis. ROC curve was constructed to permit the selection of threshold values for test results and comparison of different testing strategies.

Results

Our research involved 50 de novo AML patients, with more than half of them being males (54%), M:F ratio 1.2, and their age ranged from 18 to 72 years with a median of 44 years. Bone ache and fatigue were the most frequent presentations (82%), followed by fever (64%), bleeding (60%), purpura (58%), gum hypertrophy (32%), splenomegaly (18%), hepatomegaly (12%) and finally lymphadenopathy (10%). According to FAB classification, the most frequent type was M4 (34%), followed by M2 (30%), while M1 and M5 constitute 18% for each. Normal cytogenetic was found in 72%, while abnormal cytogenetic was found in 28% (Table 1).

PIM-2 level was assessed in different categories, and it was observed that PIM-2 level was significantly found at a lower level in the favorable cytogenetic risk group (median=1.06) versus other cytogenetic groups (median= 4) (p=0.006); also, a highly statistically significant lower PIM-2 level was observed in AML patients who achieved remission (p≤0.001). No statistically significant difference in PIM-2 level was found in patients who had early death than others, while a statistically significant higher PIM-2 level was observed in patients who suffered early relapse (p=0.02). The median of PIM-2 level in female AML patients was higher than male patients, but with no statistical significance. Regarding FAB, the median of PIM-2 expression was the highest in M1, followed by M5, then M2 and lastly M4, but with no statistical significance (Table 2).

A highly significant positive correlation between PIM-2 level and BM blast on day 28 (p<0.001, *r*=0.855) (Figure 1), also a highly significant positive correlation was found between PIM-2 level and PB blast on day 28 (p<0.001, *r*=0.856), while no significant correlation was found between PIM-2 level, age, total leukocytic count (TLC), hemoglobin level (Hb), platelets count, ESR and LDH (Table 3).

The logistic regression was used to determine the effects of various parameters on the likelihood of achieving CR. The univariate analysis revealed that response was dependently associated with TLC, cytogenetics, and PIM-2. However, on multivariable analysis, PIM-2 level was independently associated with response to therapy (Table 4).

A highly statistically significant difference was observed between patients and the control samples as regard PIM-2 expression level (p<0.001) (Table 5). Receiver operating characteristic curve (ROC) and area under the curve (AUC) for PIM-2 at diagnosis for achieving CR after induction was calculated, and it was found that PIM-2 at cutoff < 1.6 had an AUC (0.903) with a sensitivity (90.48%) and specificity (86.21%), P= <0.001 (Figure 2 and Table 6).

There was no statistically significant difference between AML patients with high PIM-2 level and those with low PIM-2 level regarding their clinical presentation (Table 7). Upon analysis of PIM-2 expression level among different

Table 1 Baseline Patient Characteristics [Median (Range) or n (%)]

		n = 50	%
Age		44 (18–72)	
Sex	F	23	46.0
	M	27	54.0
Presentation			
Fatigue	P	41	82.0
Fever	P	32	64.0
Bone ache	P	41	82.0
Gum	P	16	32.0
Bleeding	P	30	60.0
Purpura	P	29	58.0
Splenomegaly	P	9	18.0
Hepatomegaly	P	6	12.0
Lymphadenopathy	P	5	10.0
Laboratory			
FAB	M1	9	18.0
	M2	15	30.0
	M4	17	34.0
	M5	9	18.0
Cytogenetic	N	36	72.0
	P	14	28.0
TLC, $1×10^9$/L		39.0 (2.0–400.0)	
Hb, g/dl		7.4 (3.9–11.1)	
PLT, $1×10^9$/L		39 (4–131)	
ESR, mm		108 (39–149)	
LDH, U/L		764 (191–3516)	
PB blast day 0		57 (15–89)	
BM blast day 0		73 (23–94)	
BM blast day 28		10 (1–70)	
PB blast day 28		3 (0–45)	
PIM-2		2.09 (0.83–81.60)	

Abbreviations: F, female; M, male; P, positive; FAB, French American British classification; TLC, total Leucocyte count; Hb, hemoglobin; PLT, platelets; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; PIM-2, Proviral Integration of Moloney virus-2.

Table 2 PIM-2 Median (Range) Levels in Different Categories

		PIM-2 Expression		Test	P
		Median	**Range**		
Sex	F	2.61	0.86–35.50	−0.04	0.969
	M	1.40	0.83–81.60		
FAB	M1	4.20	0.90–11.18	1.5	0.626
	M2	2.60	0.83–49.50		
	M4	1.60	0.90–14.42		
	M5	4.00	0.87–81.60		
Cytogenetics Risk	Others	4.0	0.90–81.60	−2.75	0.006*
	Favorable	1.06	0.83–1.3		
ED	N	2.61	0.83–81.60	−1.29	0.194
	Y	1.13	0.90–8.70		
ER	N	1.80	0.83–81.60	−2.28	0.022*
	Y	4.10	4.00–4.20		
Remission	N	8.90	1.90–81.60	−5.43	<0.001**

Notes: *P ≤ 0.05, statistically significant; **P< 0.001, statistically highly significant.
Abbreviations: PIM-2, Proviral Integration of Moloney virus-2; F, female; M, male; FAB, French American British classification; ED, early death; ER, early relapse; N, no; Y, yes.

cytogenetic risk groups, it was found that all AML patients in the favorable cytogenetic group had low PIM-2 level. In contrast, the intermediate-risk group included 39 patients (25 had high PIM-2 expression). The adverse cytogenetic risk group included only two patients. Both had high PIM-2 level and there was a statistically significant difference between the favorable cytogenetic risk group and other groups regarding their PIM-2 expression level (p= 0.003) (Table 8). A clinical outcome of patients based on PIM-2 expression was clarified in Table 9.

Discussion

AML is a group of heterogeneous hematological malignancies characterized by clonal expansion of myeloid precursors with diminished capacity for differentiation.[21] It is triggered by genetic changes that cause hematopoietic progenitor cells to convert.[1] Molecular abnormalities are the essential events in AML development despite cytogenetic changes being the most critical independent prognostic markers.[22] Additional research into the involvement of fundamental genetic and epigenetic pathways in malignancy provides more knowledge about the mechanism of leukemogenesis in AML, suggests key prognostic standards and highlights the possible therapeutic targets.[23,24]

PIM-2 belongs to a serine/threonine kinase family, which includes three members that overlie in their function, compensate for each other and target the regulators of cell cycle and survival.[25] PIM kinases are overexpressed in both hematologic malignancies, including lymphomas, CLL and acute leukemia and solid tumors such as prostate and colon cancer.[12] HOXA9 overexpression and STAT activation in AML lead to PIM-2 upregulation as they can act as transcription factors.[26,27]

In this study, PIM-2 mRNA level was elevated in AML patients versus the control samples used to interpret our results. This agreed with what was reported by El Dahshan et al that AML patients had statistically significant higher PIM-2 expression level than their control group.[28] Tamburini et al in their study reported an expression of PIM-2 in 23 AML cases compared to its deficiency in normal CD34 positive hematopoietic cells.[16] Kapelko-Slowik et al showed that AML and ALL patients in their study had significantly higher PIM-2 expression level than the control group.[17]
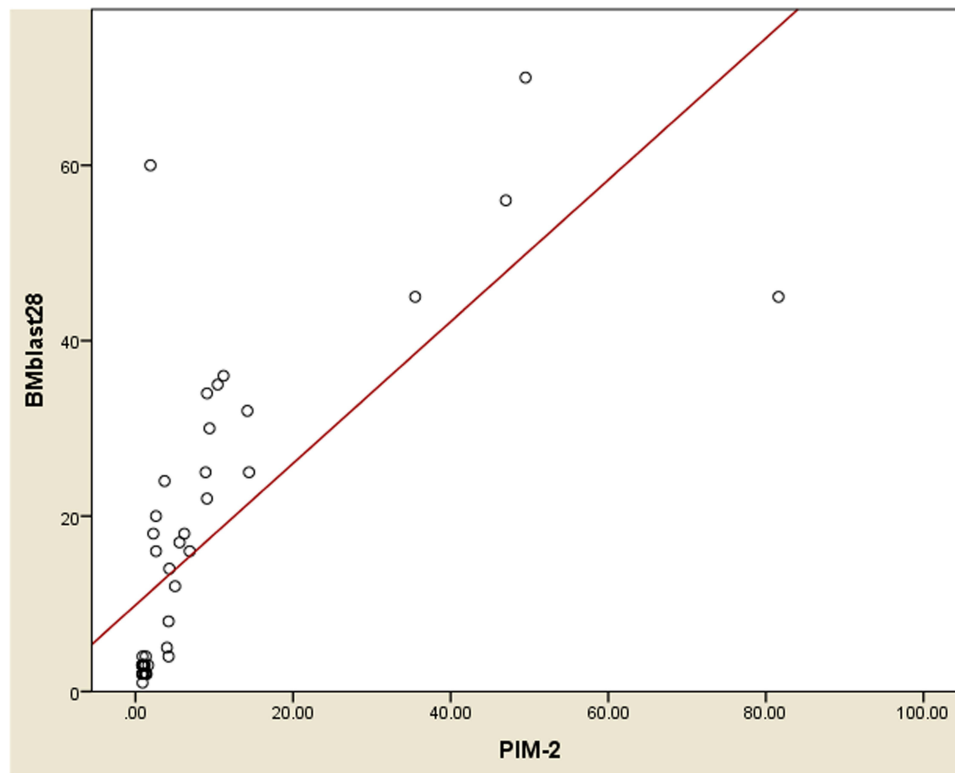
Figure 1 Significant positive correlation between PIM-2 expression and BM blasts on day 28.

Moreover, Mizuki et al compared the expression of PIM-2 mRNA in newly diagnosed AML patients' BM samples to healthy BM samples and noticed that PIM-2 mRNA was considerably higher in AML.[29]

In this research, a positive correlation was detected between PIM-2 expression level and age but did not reach statistical significance. Kapelko-Slowik et al found a significant positive correlation between these two parameters,[30] while El Dahshan et al reported a negative correlation between them but without statistical significance.[28] Regarding the

Table 3 Correlation Between PIM-2 Expression and Other Studied Parameters

	PIM-2 Expression	
	r	**P**
Age	0.237	0.097
TLC	0.163	0.257
Hb	−0.036	0.806
PLT	−0.101	0.485
PB. blast day 0	0.140	0.331
BM. blast day 0	0.177	0.218
ESR	0.044	0.764
LDH	−0.078	0.591
BM blast day 28	0.855	<0.001**
PB blast day 28	0.856	<0.001**

Notes: **P< 0.001; statistically highly significant.
Abbreviations: PIM-2, Proviral Integration of Moloney virus-2; TLC, total leucocyte count; Hb, hemoglobin; PLT, platelets; PB, peripheral blood; BM, bone marrow; ESR, erythrocyte sedimentation rate; LDH, Lactate Dehydrogenase; *r*, correlation coefficient.

Table 4 Univariate and Multivariable Logistic Regression Analyses for Response to Therapy

Covariates	Univariate			Multivariate		
	Sig.	OR	95% CI for OR	Sig.	OR	95% CI for OR
Age	0.161	0.97	0.94–1.01			
Sex (M vs F)	0.516	0.67	0.20–2.23			
TLC	0.067*	0.99	0.98–1.00	0.879	1.00	0.97–1.04
Hb	0.393	1.18	0.81–1.72			
PLT	0.998	1.00	0.98–1.02			
PB. Blasts	0.488	0.99	0.95–1.02			
BM. Blasts	0.223	0.98	0.94–1.02			
ESR	0.844	1.00	0.97–1.02			
LDH	0.414	1.00	1.00–1.00			
PIM-2	0.002*	0.26	0.11–0.61	0.005*	0.25	0.10–0.66
FAB	Reference (M1)					
M2	1.000	1.00	0.14–7.10			
M4	0.746	0.75	0.13–4.29			
M5	1.000	1.00	0.18–5.68			
Cytogenetics (Normal vs Others)	0.009*	0.14	0.03–0.60	0.398	0.35	0.03–3.98

Notes: *P< 0.1, statistically significant in univariate analysis; *P ≤ 0.05, statistically significant in multivariate analysis.
Abbreviations: OR, odds ratio; CI, confidence interval; M, male; F, female; TLC, total leucocyte count; Hb, hemoglobin; PLT, platelets; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; PIM-2, Proviral Integration of Moloney virus-2; FAB, French American British classification.

association between PIM-2 gene and different FAB subtypes, no statistically significant difference for *PIM-2* gene was found among different FAB subtypes; this agreed with Abbassy et al[31] and Kapelko-Slowik et al, who reported that no significant differences were observed between the subtypes stratified according to the FAB classification in AML.[30]

Regarding the correlation between *PIM-2* gene and different lab parameters, our results showed positive correlations between PIM-2, TLC, initial BM blasts and initial PB blasts. Still, all of them did not reach a statistically significant level. At the same time, there were negative correlations between PIM-2 and both Hb level and platelet count, but none of them was statistically significant. This agreed with Kapelko-Slowik et al, who found that no considerable correlation was observed between PIM-2 expression and Hb level, platelet count and PB blasts.[30] El Dahshan et al showed no correlation between *PIM-2* gene level and Hb or BM blast percentage. A positive correlation was noticed between the expression of PIM-2 and TLC, while a negative correlation was observed between its expression and platelet count, but with no significance.[28]

In the current study, evaluation of response to induction chemotherapy was carried out on day 28 due to BM aplasia on day 14 and poor general condition of the patients. In our study, there was a highly significant positive

Table 5 Comparison Between PIM-2 Level (Median and Range) Among Patients and Control

	AML Patients, n=50	Control, n=20	Test	P
PIM-2 level	2.09 (0.83–81.6)	0.98 (0.66–1.39)	9.4	<0.001**

Notes: **P< 0.001, statistically highly significant.
Abbreviations: AML, acute myeloid leukemia; PIM-2, Proviral Integration of Moloney virus-2.
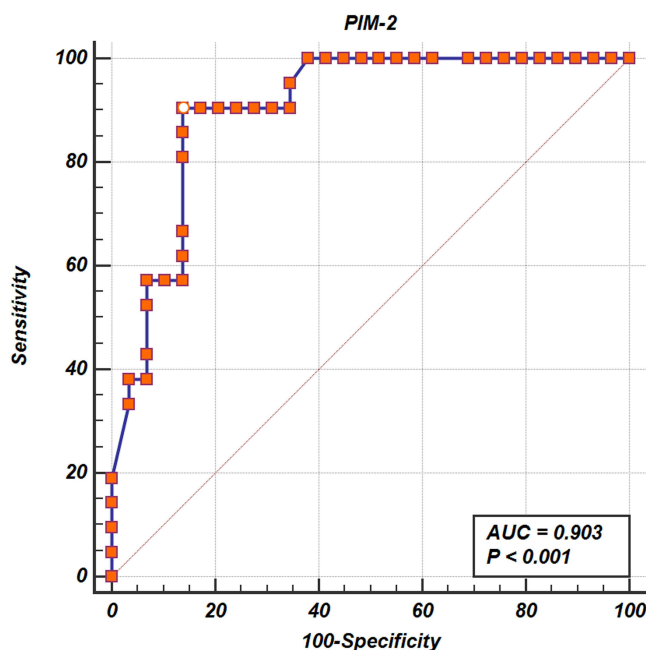
Figure 2 Receiver operating characteristic curve (ROC) and area under the curve (AUC) for PIM-2 at diagnosis (cut-off was 1.6).

correlation between PIM-2 level and BM blasts and PB blasts on day 28; also, a highly statistically significant lower PIM-2 level was found in AML patients who achieved CR than those who did not achieve. This agreed with Kapelko-Slowik et al who reported that PIM-2 gene expression was significantly lower in AML patients who achieved CR compared to those who did not respond to induction therapy.[30] El Dahshan et al noticed the same finding, but with no statistically significant difference.[28] These results denote that high PIM-2 expression level is associated with resistance to induction chemotherapy as increased PIM-2 level promotes tumorgenesis through the phosphorylation of 4E-BP1.[16] PIM-2 kinase also activates apoptosis inhibitor 5 (API-5), which is a downstream factor for Nuclear factor Kappa B (NF-KB),[32] that target many genes associated with cell survival and apoptosis inhibition such as BCL-2 and BCL-XL.[33]

In our study, the univariate logistic regression analysis for response to therapy revealed that response was dependently associated with TLC, cytogenetics, and PIM-2. However, on multivariable analysis, PIM-2 level was independently related to response to treatment and hence PIM-2 expression is an independent prognostic marker for achieving CR after standard induction chemotherapy in AML patients. Kapelko-Slowik et al in their univariate analysis to assess the survival found that the overall survival of AML patients varied according to their PIM-2 expression level.[30]

ROC curve was done for PIM-2 gene expression at diagnosis to evaluate its role in achieving remission after induction, it was found that PIM-2 at cut off value >1.6 had an AUC of 0.903 with a sensitivity of 90.48% and a specificity of 86.21%. According to this cutoff value, AML patients were alienated into two groups: low PIM-2 expression (≤1.6) and high PIM-2 expression (>1.6). As regards clinical presentation of patients based on PIM-2 expression, our results showed that no statistically significant difference in the clinical presentation of patients with high PIM-2 level than those with low PIM-2 level, this agreed with El Dahshan et al who found that there was no association between the expression level of the gene and the existence of hepatosplenomegaly or lymphadenopathy.[28]

Table 6 Receiver Operating Characteristic Curve (ROC) for PIM-2 at Diagnosis for Achieving Remission After Induction

Gene	Cut-off	Sensitivity %	95% CI	Specificity %	95% CI	AUC	SE	95% CI	Z	P
PIM-2	≤1.6	90.5	69.6–98.8	86.2	68.3–96.1	0.903	0.043	0.786–0.969	9.4	<0.001**

Notes: **P< 0.001, statistically highly significant.
Abbreviations: 95% CI, 95% confidence interval; AUC, area under the ROC curve; SE, standard error.

Table 7 Clinical Presentation of Patients Based on PIM-2 Expression

		PIM-2 Expression				Test	P
		Low, n=23		High, n=27			
		No	%	No	%		
Fatigue	N	3	13.0	6	22.2	1.7	0.429
	P	20	87.0	21	77.8		
Fever	N	9	39.1	9	33.3	0.2	0.670
	P	14	60.9	18	66.7		
Bone ache	N	5	21.7	4	14.8	0.4	0.525
	P	18	78.3	23	85.2		
Gum	N	15	65.2	19	70.4	0.2	0.697
	P	8	34.8	8	29.6		
Bleeding	N	10	43.5	10	37.0	1.8	0.4
	P	13	56.5	17	63.0%		
Purpura	N	10	43.5	11	40.	1.8	0.411
	P	13	56.5	16	59.3		
Spleen	N	19	82.6	22	81.5	0.0	0.918
	P	4	17.4	5	18.5		
Hepatomegaly	N	19	82.6	25	92.6	1.9	0.379
	P	4	17.4	2	7.4		
LN	N	22	95.7	23	85.2	4.7	0.094
	P	1	4.3	4	14.8		

Abbreviations: PIM-2, Proviral Integration of Moloney virus-2; N, negative; P, positive; LN, lymph nodes.

Our results showed that all AML patients in the favorable cytogenetic risk group have a statistically significant lower PIM-2 level than those in the intermediate and adverse groups. This data matches the findings of Kapelko-Slowik et al, who found that increased PIM-2 expression was linked to cytogenetic and molecular hazards in AML[17] and Green et al

Table 8 PIM-2 Expression Among Different Cytogenetic Risk Groups

	PIM-2 Expression				X^2	P
	Low, n=23		High, n=27			
	No.	%	No.	%		
Favorable risk group	9	39.1	0	0	13.8	0.003*
Intermediate risk group • Normal karyotyping	13	56.5	23	85.2		
• Others	1	4.3	2	7.4		
Adverse risk group	0	0	2	7.4		

Notes: *P ≤ 0.05, statistically significant.
Abbreviation: PIM-2, Proviral Integration of Moloney virus-2.

Table 9 Clinical Outcomes of AML Group Based on PIM-2 Expression

		PIM-2 Expression						Test	P
		Low, n=23		High, n=27					
		No.	%	No.	%				
ED	N	19	82.6	25	92.6			1.2	0.279
	Y	4	17.4	2	7.4				
Remission	Y	19	100.0	2	8.0			37.3	<0.001**
	N	0	0.0	23	92.0				
Responders		PIM-2 Expression				Total, n=21		Test	P
		Low, n=19		High, n=2					
		No.	%	No.	%	No.	%		
ER	N	19	100.0	0	0.0	19	90.5	11	<0.001**
	Y	0	0.0	2	100.0	2	9.5		

Notes: **P< 0.001, statistically highly significant. Remission calculated after rolling out ED, ER calculated from those who achieved complete remission.
Abbreviations: ED, early death; ER, early relapse; N, no; Y, yes.

who reported that PIM-2 was over-expressed in FLT3-ITD positive AML patients, PIM kinase inhibition reduces AML cell viability and PIM-2 knockdown blocks disease propagation in FLT3-ITD-positive AML xenografts.[34]

To investigate the impact of PIM-2 expression on the clinical outcome, our results showed that no significant association was found between PIM-2 level and mortality, while on following up the cases that achieved CR, 2 of them relapsed and it was found that they have a highly statistically significant higher PIM-2 level than those who did not relapse (p≤ 0.001). Abbassy et al had no mortality in their studied group.[31] However, Kapelko-Slowik et al in their study showed that increased PIM-2 expression in AML patients' blast cells is linked to a bad prognosis, while its lower expression resulted in prolongation of event-free survival (EFS).[17]

Finally, this study offers us an insight on *PIM-2* gene, one of the PIM kinases that plays a critical role in the leukemogenesis process and resistance to chemotherapy, assesses its expression level in AML patients in order to evaluate its role as a prognostic marker for disease outcome.

Nevertheless, the current study had some inadequacies and limitations, such as the small sample size, that it was done in single centre, the short duration of patients' follow-up and the lack of data about other genes that affect disease outcome in AML patients.

Conclusion

PIM-2 overexpression is associated with a low remission rate. PIM-2 is an independent prognostic marker for achieving CR after standard induction chemotherapy in AML patients. These results denote an association between the *PIM-2* gene and poor outcome in AML. Further long-term clinical follow-up studies with a bigger sample size are recommended to make the statistical analysis more conclusive.

Ethical Approval

All procedures involving human subjects were carried out in compliance with IRB's ethical standards (ZU-IRB# 4240). The study was performed in accordance with the Helsinki Declaration and was approved by the IRB.

Informed Consent

Informed consent was obtained from all individuals.

Disclosure

The authors report no conflicts of interest in this work.

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