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T-cell immunoglobulin mucin-3 expression levels in pediatric acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is a hematological ailment characterized via specific clinical and molecular heterogeneous disorders. It is associated with poor long-term survival, even with new chemotherapy regimens. T-cell immunoglobulin and mucin domain-3 (TIM-3) is a membrane protein expressed in various kinds of immune cells. Recent studies reported that higher TIM-3 expression levels correlate with advanced tumor stages and poor prognosis in several solid tumors. This study aimed to evaluate the expression of TIM-3 as a specific marker of leukemia stem cells (LSCs) in pediatric patients with newly diagnosed AML, and its possible role as a prognostic biomarker. The expression levels of TIM-3 were assessed in the bone marrow aspirate (BMA) of 32 newly diagnosed pediatric AML cases and 10 control subjects by flow cytometry on (CD34+/CD38+) fraction, as well as on (CD34+/CD38-) fraction, at the time of diagnosis and at the end of the first cycle of chemotherapy (first induction). These expression levels in patients were then correlated with clinical outcome. TIM-3 expression levels were significantly higher in pediatric AML patients on LSCs (CD34+/CD38-) and leukemic progenitors (CD34+/CD38+) fractions compared to the control group (p-value < 0.001). TIM-3 expression levels on LSCs (CD34+/CD38-) fraction were associated with a higher mortality risk and short survival. In conclusion, T-cell immunoglobulin and mucin domain-3 (TIM-3) may serve as LSCs specific biomarker for poor prognosis in pediatric AML patients.

Keywords: AML, leukemia stem cells, minimal residual disease, leukemic progenitors, TIM-3.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder characterized with the aid of malignant transformation of the hematopoietic stem cells (HSCs).[1] Even though ~90 % of AML cases achieve complete remission after intense chemotherapy, but an extensive proportion of AML cases (~60 %) sooner or later relapses. The recurrence of AML in those patients may be due to re-growth of surviving leukemia stem cells (LSCs).[2]

LSCs are defined as cells that are capable of starting the disease when transplanted into

immune-deficient animals and may self-renew by means of giving rise to leukemia in serial transplantations and additionally, in part differentiate into non-LSC bulk blasts that resemble the original disease, however, are unable to self-renew.[3] The identification of LSCs in AML is mainly significant in disease diagnosis, prognosis, tracking and drug screening of AML. The identity and targeting of LSCs have been relying on membrane markers, transcription factors and other unique mechanisms to selectively eradicate LSCs while sparing regular HSCs.[4] In humans, AML LSCs were originally identified in the (CD34+/CD38−) fraction, whose phenotype is analogous to HSCs.[5] However, the expression pattern of other surface molecules in the (CD34+/CD38-) fraction of AML cells is different from that of normal controls. It is known that, compared with (CD34+/CD38−) HSCs and (CD34+/CD38−) LSCs often aberrantly express cell surface markers, whose patterns may be very heterogeneous.[6] Markers expressed on LSCs include myeloid antigens CD13, CD33 and CD123 [7], CLL-1,[8] the lineage markers CD2,CD7, CD11b, CD14, CD15, CD19, CD22, CD56, CD96, and T-cell immunoglobulin mucin-3 (TIM-3).[9]

T-cell immunoglobulin and mucin domain 3 (TIM-3); (also known as Hepatitis A virus cellular receptor 2), is a membrane protein expressed on the surface of immune cells such as CD4+ T helper 1 (Th1), CD8+ T cytotoxic cells, regulatory T (Treg) cells, dendritic cells (DCs), natural killer (NK) cells, myeloid cells and mast cells.[10] Recent studies reported that TIM-3 acts as an immune checkpoint molecule that mediates T cell dysfunction or exhaustion associated with immune evasion in cancer. TIM-3 expression has been found to correlate with advanced tumor stage and poor prognosis in some solid tumors. Therefore, TIM-3 may be considered as a potential prognostic biomarker for a variety of tumors.[11]

However, the correlation between TIM-3 expression levels with AML prognosis is still controversial and eligible for further investigation. And hence, the current study aimed to evaluate TIM-3 expression levels as a specific LSC marker at the time of diagnosis (initially) and at the end of chemotherapy first induction in newly diagnosed pediatric AML patients. Then correlate these levels with the clinicopathological characteristics and clinical outcome in these patients. This could allow identifying its possible role as a prognostic biomarker as well as in targeted therapy.

Subjects and Methods

Patient and Control Subjects

This study included 32 newly diagnosed pediatric AML patients, as well as ten age, and sex matched children came to hospital for other reasons were enrolled to serve as controls. The sample size was chosen based on feasibility and experience with previous analysis.[12]

In the current study patients below 18 years old with de novo AML, were selected. However, those who have formerly received any other chemotherapy, radiation remedy, or any other anti-leukemic therapy, or patients with known bone marrow failure syndrome or another concurrent malignancy were excluded. Additionally, patients with secondary AML, relapsed AML, or unfit to receive chemotherapy were excluded. Acute promyelocytic leukemia (APL) cases were not included in the current study due to different disease biology and treatment approaches. Control subjects with any history of acute or chronic disease were excluded from the study.

The diagnosis was established according to the French-American-British (FAB) classification criteria and the World Health Organization (WHO) criteria.[13] The diagnosis was based on a detailed history, a complete clinical examination, and routine laboratory tests, including complete blood count (CBC), bone marrow aspiration (BMA), in combination with immunophenotyping (IPT) using acute leukemia diagnostic panel. The monoclonal antibodies used for the immunophenotyping included CD45, CD34, CD117, CD13, CD33, CD14, CD64, HLA-DR, MPO, CD19, CD5, CD7, CD2, CD4, and CD8. Routine lab investigations also included conventional karyotyping for detection of the common fusion genes for AML, such as t (15;17), t (8;21), inversion (16) (Inv.16), and

FMs-like tyrosine kinase-3 gene Internal Tandem Duplication (FLT3/ITD).

Specimens

Bone marrow aspirations (BMA) from patients and control subjects were taken as a part of routine investigations for diagnosis and following up. The first few drops of aspirate were used for spreading smears to be examined by using Leishman stain and cytochemical stains. Another part (2 ml) of the aspirate was withdrawn and dispensed into two K-EDTA tubes. The first one was used for routine IPT and flow cytometric detection of TIM-3 expression, and the other one for routine molecular analysis. Bone marrow aspirates (BMA) for controls were also processed for TIM-3 expression.

The antibodies used in this study were TIM-3 phycoerythrin (PE) (Clone7D3, BD Bioscience, CA, USA), CD34 fluorescein isothiocyanate (FITC) (Clone 581, Beckman Coulter, USA) and CD38 APC (Clone LS198-4-3, Beckman Coulter, USA). BMAs for cases and controls were processed within 24 hours of collection and preserved at room temperature. BMA samples of patients were collected at the time of diagnosis and at the end of induction I (day 28).

Methods

All 32 AML patients received the induction I standard chemotherapy in accordance to Acute Myeloid Leukemia guideline 2016 for newly diagnosed pediatric AML.[14] At the end of induction I, i.e., on day 28 (D28), all survived patients were evaluated for their responsiveness to treatment. Complete blood counts (CBC), BMA examination and flow cytometric minimal residual disease (MRD) analysis were all included in the evaluation.

All survived patients completed the treatment map, and were followed up for 12 months. All demographic and routine laboratory data were collected from patient files after acquiring consent from legal guardians of the patients and control subjects.

Flow Cytometric Analysis

Cell surface staining analysis for TIM-3, CD34, and CD38 was performed at the time of diagnosis for patients and control subjects and at end of chemotherapy first induction period for patients only. Two sets of 5 ml spherical bottom Falcon polystyrene tubes were labeled for the patient's first name and lab number. The first; one was used for the studied markers and the other for the isotype matched controls. An aliquot (50 µl) of count-adjusted BMA sample was placed in each of two tubes, one labeled as control and the other as test. An aliquot (5 µl) of each the following fluorescence-conjugated monoclonal antibodies (mAbs), TIM-3 PE (Clone7D3, BD Bioscience, CA, USA), CD34 FITC (Clone 581, Beckman Coulter, USA) and CD38 APC (Clone LS198-4-3, Beckman Coulter, USA) were added to the tube marked test. Isotype-matched controls were added to the control tube. Both test and control were incubated for 30 min at room temperature in the dark. A volume of 3.5 ml of ammonium chloride lysing reagent was added to each tube, inverted once and kept for 3 min. The tubes were centrifuged at 1500 xg (3200 rpm) for 3 min, then the supernatant was discarded, and the cell pellet was re-suspended. Then, 3.5 ml of phosphate buffered saline (PBS) was added to all tubes, and centrifuged at 1500 xg (3200 rpm) for 3 min. Then the supernatant was discarded, and cells were re-suspended in 500 µl PBS and cells were acquired by a flow cytometry (Navios flow cytometer, Beckman Coulter, Brea, CA, USA). 50,000 events were acquired.[15]

Flow cytometric analysis of the blast population was performed for the detection of TIM-3 expression levels on both leukemia stem cells (LSCs) (CD34+/CD38-) and leukemia progenitors (CD34+/CD38+). A cluster of 50 events was considered positive for TIM-3 expression (sensitivity 0.01). Analysis was done using the Beckman Coulter software for multi-color data (Kalousa analysis software).

Statistical Analysis

To analyze the data, the social science statistical software (SPSS version 22.0, IBM/SPSS Inc., Chicago, IL) was utilized. Descriptive statistics comprised frequency with percentage (%) for qualitative data and estimates for describing the statistical median (Med) for quantitative data. To compare the differences between two

groups of variables, the Mann-Whitney test and Spearman rank correlation coefficient were used. A *p*-value of < 0.05 indicated statistically significant data.

Results

Clinical Traits of AML Patients

Table 1 presents the clinical features and demographic information of the 32 pediatric patients with AML diagnosis.

Table 1. Demographic data and clinical characteristics of the 32 study AML patients.

Patients characteristics	
Age, median years (range)	5.5 years (30 days-16 years)
Gender, n (%)	
Male	21 (65.6%)
Female	11 (34.4%)
WBCs (10^3cell/µL) (range)	43.07 (1.2 - 312.2)
HB (g/dL) (range)	7.9 (4 – 12)
PLT counts (10^3cell/µL) (range)	47.5 (10 – 836)
Lymphocyte (%) (range)	29.5 (0 – 88)
Monocyte (%) (range)	8.5 (0 – 72)
Neutrophil (%) (range)	4.5 (0 – 37)
BM Blast % (range)	59.5 (29 – 89)
FAB, n (%)	.
M0	1 (3.1)
M1	5 (16.6)
M2	8 (25)
M4	12 (37.5)
M5	6 (18.8)
Cytogenetics, n (%)	
Normal karyotype	22 (68.75)
t(8;21)	4 (12.5)
Inversion (16)	6 (18.8)
Gene mutations, n (%)	
FLT3-ITD	6 (18.8)
NPM1	4 (12.5)

Data are presented as the median; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; BM, bone marrow; FAB, French-American-British classification; FLT3-ITD, FMs-like tyrosine kinase-3 gene internal tandem duplication; NPM1, Nucleophosmin gene.

Clinical Outcomes of The Study AML Patients

At the end of induction I period (Day 28), 22 of the 32 patients (68.75%) had complete remission (CR), and 10 patients (31.25%) died with unknown disease status. While at the end of the 12-months follow up period, 7 patients (21.9%) survived for more than one year, 6 patients (18.8%) relapsed and died before one year, and 9 patients (28.1%) died before one year with unknown disease status.

TIM-3 Expression Levels in AML Patients

TIM-3 expression levels in AML patients on (CD34+/CD38+) progenitors and (CD34+/CD38-) LSCs at the time of diagnosis and at the end of induction I period are presented in Table 2. TIM-3 showed higher expression levels (initial) on (CD34+/CD38+) progenitors compared to its expression levels on (CD34+/CD38-) LSCs, while TIM-3 showed lower expression levels on both of progenitors and LSCs fractions at the end of induction I period compared with its expression levels at diagnosis.

Table 2. T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels in acute myeloid leukemia (AML) patients at the initial diagnosis and at the end of induction I period.

TIM-3 expression level		Median (Range)
At diagnosis	TIM-3(CD34+/CD38+)%	0.76 (0.03-63.3)
	TIM-3(CD34+/CD38-)%	0.13 (0.01-3.14)
At end of induction I	TIM-3(CD34+/CD38+)%	0.64 (0.01-1.21)
	TIM-3CD34+/CD38-)%	0.07 (0-0.70)

TIM-3 Expression in AML Patients and Control Subjects

A comparison between TIM-3 expression levels in AML patients and the control group is presented in Table 3. TIM-3 expression levels were statistically significantly up-regulated in AML patients in both fractions (CD34+/CD38+) and (CD34+/CD38-) compared with that in the normal individuals (control group) (p <0.001).

Table 3. Comparison between T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels in acute myeloid leukemia (AML) patients and the control group at the initial diagnosis.

TIM-3 expression levels	AML (n= 32)	Control (n= 10)	*p-value
Initial TIM-3 (CD34+/CD38+)%			
Median	0.76	0.03	<0.001
Range (min-max)	0.03 - 63.30	0.01 - 0.08	
Initial TIM-3 (CD34+/CD38-)%			
Median	0.13	0	<0.001
Range (min-max)	0.01 - 3.14	0.00 - 0.02	

*Mann-Whitney test $p \leq 0.05$ is significant.

Association between AML Patients' Clinicopathological Features and TIM-3 Expression

Due to the wide range of ages, the median was used for statistical purpose. Patients were divided into two groups, a young group with age ranged from 1 month to 5.5 years, and an old group with age ranged from 6 years to 16 years. It was found that the young group expressed statistically significantly higher levels of TIM-3 on leukemic progenitors at diagnosis with a median (3.65%) than the old group with a median (0.51%) (p = 0.04). However, there was no significant statistical association between the initial expression levels of TIM-3 on LSCs with age, as shown in Table 4.

Table 4. T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels at diagnosis time in two age groups of acute myeloid leukemia (AML) patients.

Age group	Young (n= 16)	Old (n= 16)	*p-value
Initial TIM-3 (CD34+/CD38+)%			
Median	3.65	0.51	0.04
Range (min-max)	0.025 - 63.30	0.077 - 51.04	
Initial TIM-3 (CD34+/CD38-)%			
Median	0.139	0.066	NS
Range (min-max)	0.0059 - 3.14	0.0079 - 1.78	

*Mann-Whitney U test $p > 0.05$ is not significant (NS).

No significant statistical association was reported between the initial expression levels of TIM-3 on both fractions (CD34+/CD38+) and (CD34+/CD38-) with gender, WBCs, HB levels, PLT counts, lymphocyte (%), monocyte (%), and neutrophil (%). However, there was a statistically significant positive relation between initial TIM-3 expression levels on leukemic progenitors (CD34+/CD38+) fraction and bone marrow (BM) blast (%) (p = 0.047). This relation was not found between LSCs (CD34+/CD38-) and BM blast (%), as presented in Tables 5.

There was no statistically significant association seen between the initial expression levels of TIM-3 and any of the AML FAB subtypes in both fractions. Similarly, no statistically significant association was reported between initial TIM-3 expression levels on both fractions (CD34+/CD38+) and (CD34+/CD38-) and molecular markers (t (8;21), Inv.16, FLT3/ITD, Nucleophosmin gene 1(NPM1) mutations.

Table 5. Correlation between T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels at the initial diagnosis time and hematological parameters in acute myeloid leukemia (AML) patients.

Parameters correlated	Initial TIM-3 (CD34+/CD38+)%		Initial TIM-3 (CD34+/CD38-)%	
	r	*p*-value	r	*p*-value
WBCs (10^3 cell/µL)	0.05	NS	0.02	NS
Lymphocyte (%)	0.35	NS	0.21	NS
Monocyte (%)	0.11	NS	0.08	NS
Neutrophil (%)	0.15	NS	0.03	NS
Hemoglobin (g/dL)	-0.07	NS	-0.18	NS
Platelets counts (10^3 cell/µL)	0.11	NS	0.00	NS
BM Blast % (range)	0.35	0.047	-0.11	NS

r : Spearman correlation coefficient $p > 0.05$ is not significant (NS).

TIM-3 expression and AML patients' clinical outcomes

To evaluate the impact of TIM‑3 expression levels on the clinical outcomes of AML patients in terms of their responsiveness to induction I chemotherapy and the overall one year survival, patients were categorized into two groups. Low and high TIM-3 expression groups were assigned based on the median values. By the end of the first induction cycle of treatment, on Day 28 (D28), 10 patients representing (31.2%) died before the first induction cycle ended. Regarding the median of their initial TIM-3 expression level it was found that, on leukemic progenitors (CD34+/CD38+) fraction, a total of 5 patients (15.65%) in the low TIM-3 expression group died, and 5 patients (15.65%) in the high TIM-3 expression group died. While on LSCs (CD34+/CD38-) fraction, a total of 3 patients (9.4%) in the low TIM-3 expression group died, and 7 patients (21.9%) in the high TIM-3 expression group died (Table 6).

Table 6. T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels at the initial diagnosis time on leukemic progenitors (CD34+/CD38+) and on leukemia stem cells (LSCs) (CD34+/CD38-) and post induction (I) clinical outcome in acute myeloid leukemia (AML) patients.

Initial TIM-3(CD34+/CD38+) %	Low group	High group
Median	≤ 0.76	>0.76
No of cases	(n= 16)	(n= 16)
Survival on D 28 [n (%)]		
Alive	11 (68.8)	11 (68.8)
Dead	5 (31.3)	5 (31.3)
Initial TIM-3 (CD34+/CD38-) %		
Median	≤ 0.13	> 0.13
No of cases	(n= 16)	(n= 16)
Survival on D28 [n (%)]		
Alive	13 (81.3)	9 (56.3)
Dead	3 (18.8)	7 (43.8)

The 22 survived patients (68.8%) were followed up for 12 months. At the end of the follow up period only 7 patients (31.8%) survived and 15 patients (68.2%) died before the end of the follow-up period. Regarding the median of their end of induction I TIM-3 expression levels, it was found that, on leukemic progenitors (CD34+/CD38+) fraction, a total of 7 patients (31.8%) in the low TIM-3 expression group died, and 8 patients (36.4%) in the high TIM-3 expression group died. While on LSCs fraction a total of 5 patients (22.7%) in the low TIM-3 expression group died, and 10 patients (45.5%) in the high TIM-3 expression group died (Table 7).

Table 7. T-cells immunoglobulin and mucin domain 3 (TIM-3) expression levels at the end of induction (I) on leukemic progenitors (CD34+/CD38+) and on leukemia stem cells (LSCs) (CD34+/CD38-) and clinical outcomes in acute myeloid leukemia (AML) patients at a 1-year follow-up period.

End of induction I TIM-3 (CD34+/CD38+) %	Low group	High group
Median	≤ 0.64	>0.64
No of cases	(n= 11)	(n= 11)
Survival on 1 year [n (%)]		
Alive	4 (36.4)	3 (27.3)
Dead	7 (63.6)	8 (72.7)
End of induction I TIM-3 (CD34+/CD38-)%		
Median	≤0.07	>0.07
No of cases	(n= 11)	(n= 11)
Survival on 1 year [n (%)]		
Alive	6 (54.5)	1 (9.1)
Dead	5 (45.5)	10 (90.9)

Discussion

Several studies have reported that AML relapse and chemotherapy resistance may arise from small clones, known as LSCs [16]. The identification of LSCs in AML is mainly significant in disease diagnosis, prognosis, tracking and drug screening of AML. The identity and targeting of LSCs have been relying on the membrane surface markers to selectively eradicate LSCs while sparing regular HSCs.[17] Also, LSC surface markers can be used to monitor minimal residual disease (MRD) by flow cytometry.[18]

TIM-3 is a membrane protein expressed in various kinds of immune cells. Recent studies reported that higher TIM-3 expression levels have been found to correlate with advanced tumor stage and poor prognosis in some solid tumors.[19, 20, 21, & 22]

In the present study, the expression level of TIM-3 was simultaneously assessed on LSCs and leukemic progenitors in BM of newly diagnosed pediatric AML patients. The results showed that TIM-3 expression levels were significantly higher in AML patients on leukemic progenitors (CD34+/CD38+) and LSCs (CD34+/CD38-) fractions compared with those in the control group (p <0.001).

The study by Kikushige 2021, [5] reported that the human gene hepatitis A virus cellular receptor 2, the gene that encodes TIM-3 in human, was highly expressed on LSCs but not on normal HSCs due to genetic mutation. Both HSCs and LSCs can be evaluated for their expression. It was reported that TIM-3 is expressed on both LSCs and 1eukemic progenitors fractions of AML, but its expression tended to decline at the (CD34-) fraction and not expressed by normal HSCs (CD34+/CD38-) or the vast majority of normal progenitors (CD34+/CD38+) fraction. Similarly, the study by Haubner *et al.,* 2019[23] and Kamal *et al.,* 2021,[24] found that TIM-3 expression is up-regulated on the LSCs and on the majority of their downstream CD38+ (leukemic progenitors) in most AML subtypes except for APL compared with that in the control group.

In the present work, TIM-3 showed higher expression levels on leukemic progenitors (CD34+CD38+) fraction compared with their expression levels on LSCs (CD34+/CD38-) fraction at the time of diagnosis and at the end of induction I time (Day 28). Also, TIM-3 showed decrease in its expression levels on leukemic progenitors and LSCs by the end of induction I time compared with their expression levels at the time of initial diagnosis. These results agreed with those of Xu *et al.,* 2017,[20] who also, found that TIM-3 expression on AML blast significantly decreased after complete remission compared to that in the initial diagnosed AML patients. Also, the study by Mohamed *et al.,* 2021, [25] reported that the levels of LSCs (CD34+/ CD38–/ TIM3+) frequency at the initial diagnosis time were significantly higher compared with their post induction expression levels.

The results of the current study observed that there was a significantly higher expression of initial TIM-3 on leukemic progenitors (CD34+/CD38+) among the younger age group (p=0.04). This association was not found between initial TIM-3 expressions on LSCs and patients ages. Conversely, Kamal *et al.,* 2021, [24] and Wang *et al.,* 2022,[21] found no correlation between TIM-3 gene expression levels in adult AML patients and patient's ages. The reason for the discrepancy in the results may be traced back to the age range of the study group in two previous studies and this current work.

In the present work, there was no significant association between TIM-3 expression levels and gender or molecular mutations (inversion (16), t (8;21), FLT3/ITD and gene NPM1 gene mutations). The results of this study are in concordance with those of Wu *et al.,* 2022,[26] who found that TIM-3 expression levels were not affected by gender, or molecular mutations.

The present study observed that there was no significant association between TIM-3 expression levels on both fractions with hemoglobin concentration, WBCs, or platelet counts of AML patients. These agreed with those of Hong *et al.,* 2022,[22] who observed that TIM-3 expression levels on leukemic blasts were not significantly correlated with CBC results. On the other hand, Tasneem *et al.,* 2019,[12] noticed that CD34+/CD38+/TIM3+ had significant correlations with WBCs, and platelets count, but CD34+/CD38-/TIM3+ had insignificant correlations with other laboratory data.

In the present work, there was a significant positive relation between TIM-3 expression levels on leukemic progenitors (CD34+/CD38+) fraction and the percentage of BM blast (p =0.047), while this correlation was not significant for LSCs fraction. These results were in accordance with those of Tasneem *et al.,* 2019,[12] who noticed that CD34+CD38+TIM3+ had significant correlations with BM blast, while CD34+/ CD38-/ TIM-3+ had no significant correlation with BM blast.

Regarding FAB classification in our study, there was no association between TIM-3 expression levels on both fractions and FAB subtypes for AML patients. The study by Herrmann *et al.,* 2020,[27] performed molecular analysis and applied a larger panel of monoclonal antibodies to establish and compare aberrant surface antigens in various AML groups. The study observed that the LSCs (CD34+/CD38-) and leukemic progenitors (CD34+/CD38+) in AML had considerable heterogeneity without a definitive relationship to FAB or the WHO variants.

The results of the present study demonstrated that high TIM-3 expression levels on LSCs (CD34+/CD38-) were associated with a higher mortality risk and shorter survival. However, this association was not found with leukemic progenitors (CD34+/CD38+). The study by Wang *et al.,* 2017,[28] found that only the LSCs (CD34+/CD38−) could initiate leukemia in immune-deficient mice. Additionally, it has been demonstrated that these LSCs, with highly expressed adenosine triphosphate binding cassette transporters, were shown to protect themselves from the attacks from chemotherapeutic agents.[28]

TIM-3 is considered as a negative regulator of T-cell driven immune response as it plays an essential role in the innate and cellular immunity and immune tolerance. After binding to its main ligand galectin-9, TIM-3 induces Th1 cells to undergo apoptosis and inhibits the production of interferon -γ, which inhibits the activity of T-cells and thereby contributes to immune escape.[29] Moreover, both TIM-3 and its ligand Galectin-9 constitute an autocrine loop that induces the activation of the nuclear factor-kB (NF-κB) and β-catenin signaling, which promotes gene expression modifications leading to improved LSCs survival.[25] Based on these previously mentioned data the study by Wu *et al.,* 2022,[26] demonstrated that TIM-3 has a dual role in AML. The first TIM-3 expressed on LSC (CD34+/CD38−) promotes leukemogenesis, and second TIM-3 expressed in immune cells leads to immune dysregulation. Therefore, in addition to mediating LSC-related signaling pathways, TIM-3 also affects the efficacy of AML treatment through immune responses. These results are comparable with those reported by Tasneem *et al.,* 2019, [12] who found that the patients who achieved complete remission have significantly lower (CD34+/CD38-/TIM-3) expression, more survival and more disease free survival (DFS) compared to patients who have higher (CD34+/CD38-/TIM-3) expression levels. Yao *et al.,* 2018,[30] and Mohamed *et al.,* 2021,[25] reported that AML patients with high expression levels of TIM-3 on LSCs (CD34+/CD38−) have shorter overall survival and shorter DFS compared with patients having lower expression of TIM-3 on LSCs.

In conclusion, TIM-3 was significantly expressed in AML patients but not on normal HSCs; therefore, TIM-3 could be used in AML as a specific AML biomarker. In addition, overexpression of TIM-3 on LSCs (CD34+/CD38-) was associated with risk of higher mortality and shorter survival. This suggests that TIM-3 may serve as a biomarker of poor prognosis in AML, monitor MRD, predict relapse, and it may play a possible role in targeted therapy.

Author Contributions

MAE; helped in the formulation of the main idea of the research, Revising the results and the discussion, Helped in writing the paper. ENE; Helped in examining the patients, Providing the clinical data, Performed the clinical assessment. MSMI; Helped in formulation of the idea, Helped in laboratory work and its interpretation, Helped in data analysis, Helped in writing the paper. MA; Revising the results and the discussion, Helped in writing the paper, Conducted the corrections, and language editing. OFAD; Helped in laboratory work and its interpretation, Helped in data analysis. MSM; Gathering references for the scientific background, Collected the samples, Performed the laboratory work, Drafted the paper

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by Research Ethics Committee of the Faculty of Medicine, Suez Canal University, Egypt (Research # 5209, approval dated May 2023).

Informed consent

Informed consent was obtained from the legal guardians of the patients and control subjects before taking any data or doing any investigations.

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